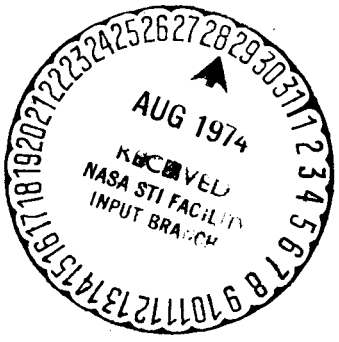
THIRD ANNUAL PROGRESS REPORT

GRANT NO. NGR.05-007-221



July 1, 1971 to September 15, 1972

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

OSSA - EXOBIOLOGY BIOSCIENCE PROGRAM

NATURE AND CHANGES OF ORGANIC MATTER IN DEEP OCEAN CORES

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INTRODUCTION

Funding for this project began in 1969. In the first year, many of the techniques were established and equipment was calibrated and tested. Studies were undertaken in collaboration with J.W. Smith on the removal and identification of carbon compounds in several carbonaceous meteorites. The results do show that in type I and type II chondrites, the bulk of the carbon must be indigenous, whereas in type III and non-carbonaceous chondrites, volatile extractable organic molecules are probably derived from contamination. The criteria were established by comparing C^{13}/C^{12} ratios in compounds removed from the meteorites and in compounds extracted from terrestrial sediments.

These criteria were tested shortly after publication of the data, through analysis of the Murchison meteorite which fell in Victoria, Australia in September, 1969. The study carried out in collaboration with Ames Research Center, gave strong support for the extraterrestrial origin of the hydrocarbons and amino acids. The C^{13}/C^{12} data gave strong support to other data on the distribution pattern of these compounds, and indicated that they could not have originated from recent terrestrial contamination of known composition. We believe this finding may be one of the most important to have been published in the field of biological studies on meteorites. It now opens new avenues of endeavor into investigation of extraterrestrial samples.

A study was also undertaken in collaboration with Mrs. Mary Jo Baedecker to investigate the origin of some isoprenoid hydrocarbons in sediments from the hypersaline lake, the Dead

Sea. These hydrocarbons, the best-known being phytane (2, 6, 10, 14-tetramethylhexadecane) and pristane (2, 6, 10, 14-tetramethylpentadecane) are very common in ancient sediments. They are often considered to be derived from cleavage of the phytol side chain of the chlorophyll molecule, and have therefore frequently been used as evidence for biogenicity in ancient rocks. Strangely enough, however, the hydrocarbon phytane is very rare in recent sediments, and pristane has been found only in trace quantities. Chlorophyll begins to degrade rapidly in these sediments. In the Dead Sea, however, chlorophyll degrades much more slowly but phytane as well as pristane are present in the surface sediment in a significant ratio to the total hydrocarbon content of the sediment. One possible source for the hydrocarbons could be the red halophilic bacteria which dominate the biota of the water column. Since it is known that these organisms contain an ether-linked glycerol-diphosphatidyl lipid, it was considered that cleavage of the linkages would release phytol which would eventually be reduced into the isoprenoid hydrocarbon. A test for this lipid in the sediment showed it was present. The isoprenoid hydrocarbons most probably arose from this source rather than a chlorophyll side chain. This fact must therefore be kept in mind when considering the origin of molecules in ancient rocks in an attempt to find evidence for initiation of a photosynthetic mechanism. A detailed report of the study is enclosed.

During 1970-1971, the sediment from five deep sea cores were examined, representing fifteen individual samples. Some of the sediment was typical of normal deep sea clays, low in organic matter

($\leq$ 0.2% organic C), whereas others were richer and approached more closely near shore environments, containing about 1.0% organic carbon. Two cores originated in the Gulf of Mexico, and the influence of upward migration of petroleum can be noticed from the unusually high hydrocarbon values. The cores of others came from the west Atlantic Ocean. The samples represent an age spread Pleistocene ($\leq$ 100,000 years) to Eocene ($\geq$ 50 X 106 years).

During the year, a search was also made to detect compounds which were specific indicators for land or marine origin. The five-ringed polynuclear hydrocarbon, perylene, was found to be a very good index. Its presence depends on a source of land-derived quinoid precursors, which are preserved under reducing conditions only. This compound, and C^{13}/C^{12} isotope values proved important indicators.

Several experiments were also carrier out on the pigments of blue-green algae and C^{13}/C^{12} values of algal mats and algal cultures grown under different conditions. It was hoped to learn from these experiments what the effects of heating was on preservation of cell morphology and cell biochemistry. In addition, it was hoped to learn more about properties of primitive cells grown under conditions which may have existed in the Precambrian.

The studies mentioned above have been written up in greater detail in the enclosed manuscripts.

More recently, a series of studies were initiated to determine changes which occur in sediments during lithification. The first experiments have been undertaken by heating very young sediment from a bay (Banderas Bay) in the southeast Gulf of

California, which is known to have a large terrigenous component in the organic matter. The carbon content of this sediment is 1.6%. The aim of the work was to follow the fate of lipids and pigments by exposing the sediment to elevated temperatures (up to 150°C) for 7 days.

Two sets of simulated maturation experiments were performed, namely the "open" vessel and the sealed ampoule method in which wet (natural) and freeze-dried samples were used. An increase of 40% in hydrocarbons could be observed in a sample which has been exposed to 150°C for one week, in both normal alkanes as well as the iso- and branched ones. Normal alkanes were depleted in the freeze-dried samples. A series of biologically-significant alkanes such as pristane (C_{19}) and pyrtane (C_{20}) were identified in the natural sediment, the quantity of these and other branched alkanes however, were increased considerably in the heat-treated samples. The distribution of n-alkanes in the wet heat-treated sediment remained unchanged, whereas they were altered in the freeze-dried samples.

Small amounts of free fatty acids were identified in all samples, however, the quantities were greater in the heat-treated samples. The acids identified were predominantly even carbon numbered (mainly C_{16}). An exception was the freeze-dried sample, heated in an open vessel which showed an even distribution of acids from C_{14} to C_{24}. Two types of hydrolyzable fatty acids were identified, those bound to simple molecules such as alcohols which can be eluted from silicic acid with benzene and those bound to more complex molecules which must be eluted from silicic acid only with

methanol. The acids identified in the untreated sediment were of the second type, saturated acid (C_{16}) being the most abundant, accompanied by C_{16} and C_{18} unsaturated acids. The amount was significantly lower in the wet heat treated sample (about 25% of the original). The freeze-dried heat-treated sample showed a slight increase in total acids, including acids of the first type (from benzene eluent). The pattern was significantly different: 1. C_{14} was the most abundant acid followed by C_{16} and C_{18}: 2. No predominance of even-over-odd was observed in acids greater than C_{18}: 3. No unsaturated C_{16} and C_{18} acids were found.

Of particular interest was the identification of resin acids in these samples. The content was increased three-fold in the wet heat-treated sample over its original content, but was very depleted in the freeze-dried samples. Although β-carotene could be detected in the original sediment, it should be pointed out that its absorption range in the UV-visible overlaps some of the aromatic compounds, especially perylene. We therefore, re-chromatographed the yellow fraction, thus separating perylene from β-carotene. The fact that perylene and other polycyclic compounds survive under conditions that β-carotene degrades, suggests that these compounds are thermodynamically favored and may be π-complexed to clays. A decrease of about 90% in the content of chlorins was observed in a sample heated at 100°C, followed by a total loss at 150°C. No porphyrins were detected, and an extractable brown-red "pigment" was probably of a polymeric-type.

In the open vessel experiment, the extractable lipids were depleted relative to aromatics and pigments; this might be due

to "steam distillation" of the interstitial water through the sediment.

Papers Published Under Support of Grant

1. J.W. Smith and I.R. Kaplan. Endogenous Carbon in Carbonaceous Meteorites. Science, 167, 1367-1370 (1970).

2. I.R. Kaplan and M.J. Baedecker. Evidence for Phosphatidyl Glycerophosphate Lipid in Hypersaline Sediment from Dead Sea. Israel J. of Chemistry, 8, 529-533 (1970).

3. K. Kvenvolden, J. Lawless, K. Pering, E. Peterson, J. Flores, C. Ponnamperuma, I. Kaplan and C. Moore. Evidence for Extraterrestrial Amino-Acids and Hydrocarbons in the Murchison Meteorite. Nature, 228, 923-926 (1970).

Papers In Press or Submitted for Publication

Distribution and diagenesis of organic compounds in JOIDES sediment from Gulf of Mexico and Western Atlantic. Z. Aizenshtat, M.J. Baedecker and I.R. Kaplan (submitted to Geochimica et Cosmochimica Acta).

Growth pattern and C^{13}/C^{12} isotope fractionation of Cyanidium caldarium and hot springs algal mats. J. Seckbach and I.R. Kaplan (submitted to Ecology).

Perylene and its Geochemical Significance. Z. Aizenshtat (In Press - Geochimica et Cosmochimica Acta).

Thermal degradation of blue-green algae and blue-green algal chlorophyll. J.H. Oehler, Z. Aizenshtat and J.W. Schopf (submitted to Geochimica et Cosmochimica Acta).

DISTRIBUTION AND DIAGENESIS OF ORGANIC COMPOUNDS IN JOIDES SEDIMENT FROM GULF OF MEXICO AND WESTERN ATLANTIC

by

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ABSTRACT

Fifteen sediment samples were studied from five drill sites recovered by the "Glomar Challenger" on Legs I and IV in the Gulf of Mexico and western Atlantic. This study concentrated on compounds derived from biogenic precursors, namely: (1) hydrocarbons, (2) fatty acids, (3) pigments and (4) amino acids. An attempt was made to determine diagenetic changes, by monitoring the carbon content of various extractable and non-extractable fractions.

Carbon isotope (δC^{13}) data (values < -26 ‰ relative to PDB), long-chain n-alkyl hydrocarbons ($> C_{27}$) with an odd-to-even preference, and presence of perylene, proved useful indicators for the terrigenous origin of the organic matter in some samples. It was found that land-derived organic matter can be transported for distances over 1,000 Km into the ocean and their source still recognized.

INTRODUCTION

Until 1968, when the deep-sea drilling and coring project was initiated by JOIDES, organic geochemical studies on unlithified marine sediments were restricted to samples buried to less than 50 m depth. This work describes the analysis of fifteen samples from five drill-sites on Legs I and IV of the JOIDES program. The deepest sample was cored from 534 m (Hole 3) and is Miocene in age.

Other analyses of JOIDES sediment samples have been reported by Koons (1970), Simoneit and Burlingame (1971), Simoneit *et al.* (1972a, b), McIver (1971, 1972), Baker (1970) and Wehmiller and Hare (1972). Generally, these have each dealt with a restricted group of compounds. Here, an attempt was made to analyse hydrocarbons, fatty acids, pigments (chlorins and porphyrins) and amino acids. The C^{13}/C^{12} ratio was measured on the organic carbon of all sediment samples, and on some extractable and non-extractable residues (e.g., humic acid and kerogen).

Porphyrins were recognized in some samples, but not in others. The five-ringed aromatic hydrocarbon perylene was found in samples originating from land-derived organic matter which had been preserved under reducing conditions.

The study was primarily designed to investigate: (i) the sources of the organic matter present in the sediment, (ii) their stability with time of accumulation and (iii) the conditions necessary for *in situ* formation of new compounds.

EXPERIMENTAL

Sample and site description

A description of the site location, water depth, depth of core penetration below the sediment-water interface, stratigraphic age, a brief lithological description, organic carbon content and δC^{13} in parts per mil, relative to the PDB scale, are given for each sample in Table 1. Detailed descriptions for each core are given in the Initial Reports of the Deep Sea Drilling Project (Ewing et al. 1969; Bader et al. 1970). Some additional notes are given below, for the purpose of discussion and interpretation of the data obtained in this study. The site locations are shown in Figs. 1 and 2.

Hole 2

Hole 2 was drilled on the Challenger Knoll salt dome. Oil was found in core 5 at 136-138 m depth within porous calcite and gypsum layers. The top sample studied (20 m depth) contained abundant plankton remains and traces of pyrite. The lower sample (103 m) had a strong smell of H_2S when opened. There was no obvious evidence for oil migration.

Hole 3

This site is about 21 nautical miles S.E. of Hole 2. The sediment consists of interbedded turbidites and normal pelagic sediment sequences. The surface cores were reported to be "silty to very silty terrigenous clays, with or without

admixture of nannoplankton and foraminifera". A fragment of fossil wood was actually detected at depth.

Evidence of pyrite in the sediment and sulfate reduction in the interstitial water (Kaplan and Presley 1969) indicates that this was a reducing environment.

Holes 6A and 6

These sites are in deep water on the west flank of the Bermuda Rise. The older Eocene sediments are dominated by turbidity flows. These grade upward to more normal pelagic sediments containing a variety of microfossil remains and volcanigenic debris. The rate of sedimentation was very slow (<0.3 cm/10^3 yrs.), allowing oxidation to proceed at the sediment surface.

Hole 26

The site for drilling is the Vema Fracture Zone (Fig. 2), a narrow east-west trending trough which cuts through the Mid-Atlantic Ridge. The location has a high heat flow (often associated with mid-ocean ridges). Rapid sedimentation has taken place as apparently all the samples cored (to 483 m) are Quaternary in age. The sediment appears to be turbidites, showing graded bedding with sand at the bottom and clay at the top of each sequence. Detrital silicates, quartz, metal oxides and heavy minerals indicate rapid transport--possibly from the Amazon River (about 1,100 Km away). Terrigenous plant fragments were recognized in the sand layers. The sediment was reducing and showed marked sulfate reduction.

Hole 27

The uppermost sample (143 m) consists of silty clay containing glauconite and pyrite. The two bottom samples were sampled in, or adjacent to, recognizable turbidites displaying graded bedding and containing plant fragments. The sediment was reducing (Presley and Kaplan, 1970), and the lower two samples showed most pronounced sulfate reduction.

Sample Storage

Sediment samples received from the Glomar Challenger had been squeezed to remove interstitial water, wrapped in aluminum foil, and frozen ; 150 g samples from each location were analyzed.

Analytical Procedures

The total organic carbon was determined after reacting a known weight of sediment with 0.2N HCl to remove carbonate. The residue was washed repeatedly with distilled water to remove all the acid and dried in a vacuum dessicator at 40°C. The dried sample was combusted in an oxygen atmosphere at 1100°C. and the volume of CO_2 measured to calculate the amount of organic carbon in the sediment. The CO_2 was collected and the C^{13}/C^{12} ratio measured on a Nuclide Co. 60° radius mass spectrometer.

A procedure was developed for each sample to determine: hydrocarbons, fatty acids, alcohols, chlorins and porphyrins. In addition, amino acids, fulvic acids, humic acids and kerogen were isolated in samples from Hole 26. A summary of the methods

used is shown in Figure 3. A detailed description of the extraction procedure is given in Brown et al. (1972). Techniques for the separation and analysis of free fatty acids, hydrolyzable fatty acids, alcohols and humic and fulvic acids have been described elsewhere (Nissenbaum et al. 1972a). Kerogen was demineralized by successive treatments with HF, HCl and HNO_3 according to the method developed by Saxby (1970). Some of the techniques used for the analysis of hydrocarbons, pigments and amino acids were modified slightly for this study.

The hexane fraction from the silicic acid column (Fig. 3) which contained the hydrocarbons, was evaporated to near-dryness and rechromatographed to separate aliphatic and aromatic hydrocarbons. The total hydrocarbon fraction was applied to a small florisil column and eluted with hexane and then with benzene. The hexane eluent containing aliphatic hydrocarbons and the benzene eluent containing aromatic hydrocarbons were analyzed by gas chromatography. Perylene was analyzed following the procedure of Aizenshtat (1972).

Part of the benzene fraction from the silicic acid column was used for the analysis of acids and alcohols (for methods, see Brown et al. 1972) and that remaining was used for the study of pigments. Porphyrin and chlorin pigments were found in the benzene and methanol fractions, respectively, of the silicic acid column. Occasionally, some chlorins appeared with the porphyrins, in which case the two fractions were recombined and chromatographed on a silica gel column. The following solvents were used for eluting the pigments: (1) hexane, (2) hexane:benzene (1:1), (3) hexane:benzene (1:4),

(4) benzene and (5) 5-20% chloroform in benzene. Porphyrins were in fractions (3) and (4); the latter contained compounds more substituted in carbonyl groups. Fraction (5) contained the chlorins. The fractions were evaporated to dryness, taken up in benzene and analyzed by ultraviolet and visible spectrometry.

The porphyrin results were calculated using the Soret peak at 390-408μ and the extinction coefficient of 4×10^5 (Hodgson, et al. 1968). The data for chlorins were calculated using the 660μ peak and $\varepsilon = 3 \times 10^4$ (Geodheer 1966).

The sediment after benzene-methanol extraction was refluxed with 6N HCl for 12 hours to hydrolyze the amino acids, and then filtered. The filtrate was concentrated by rotary evaporation under vacuum, and then diluted with 150 ml water. To this solution 3N HF was added, and while stirring, 4N LiOH was added to bring the pH in the range of 7-9 to precipitate salts. The mixture was filtered and the filtrate, containing the amino acids, was concentrated. The amino acid solution was then applied onto cation exchange resin to complete the desalting procedure. The column was washed with water and, when the eluent was chloride-free, the amino acids were eluted with 2N NH_4OH. The fractions containing amino acids were concentrated and run on an amino acid analyzer. Optical isomers were determined by gas chromatography on the N-trifluoroacetyl-(+)-2-butyl ester derivatives of the amino acids (Kvenvolden et al. 1969).

Reagents and Instrumentation

Organic solvents used in the procedure were distilled and the level of solvent contamination determined as $< 4 \times 10^{-11}$ g/ml for all solvents. The chromatographic packing materials: silicic acid (minus 325 mesh), florisil (minus 100 mesh) and silica gel

(30-60 mesh), were prewashed with the eluting solvents in reverse order of actual use and oven-dried. The cation exchange resin, AG 50W-X8, was washed before use with NaOH, water, HCl and again with water until chloride-free.

Gas chromatographic analyses were carried out on Varian Aerograph Models 204, and 1520 and Hewlett-Packard Model 5750 gas chromatographs. The following columns were used: 5'X 1/8", 3% SE-30 on 100-120 mesh Chromosorb W: 5' X 1/8", 2% Apiezon L on Chromosorb W; 5' X 1/8", 3% DEGS on Chromosorb W; and 100' (0.1" I.D.) capillary column coated with Apiezon L. Ultraviolet and visible spectra were obtained on a Cary 15 spectrophotometer, equipped with a 0.1 cc microcell. A 21-491 CEC mass spectrophotometer, coupled with a Varian model 1200 gas chromatograph employing a 5' X 1/8", 5% SE-30 column, was used for structure determinations.

RESULTS

Hydrocarbons

Hole 2

Sample 2-20 contains 17 ppm and sample 2-103 contains 4 ppm of identifiable n-alkanes. The distribution is bimodal, about 97% of the identifiable hydrocarbon is in the range C_{25}-C_{33}, (Fig. 4) with C_{29} and C_{31} accounting for at least 60%. The remainder fall in the short-chain length C_{14}-C_{19}, with no evident odd-to-even preference, although n-C_{17} predominates. For the range C_{21}-C_{33}, the CPI = 3.9 in sample 2-20 and 4.6 for sample 2-103. The isoprenoid hydrocarbons pristane and phytane were detected in low abundance (50 ppb) and the ratio pris./phyt. = 0.7 for 2-20 and 0.8 for 2-103 (see Fig 4a).

Comparison of hydrocarbons extracted from these samples with hydrocarbons from the petroleum at depth of 136-138 m in the same hole, shown in Fig. 4c (David and Bray, 1969), indicates a lack of long-chain hydrocarbons in the oil. It is therefore unlikely that the petroleum was a source of the hydrocarbons in the overlying sediment. This is further supported by a lack of aromatic hydrocarbons in the sample analysed, in comparison with the petroleum fraction.

Hole 3

The hydrocarbon pattern for the uppermost sample 3-34, is different from the three lower samples (Fig. 5). Samples 3-209, 3-324 and 3-534 contain 0.6, 1.1 and 1.0 ppm saturated n-alkyl hydrocarbon, whereas 3-34 contains 9.3 ppm. Furthermore, the lower samples have a distribution similar to those in Hole 2, with 80% identifiable hydrocarbons having a chain length $>C_{24}$, whereas 3-34 has 50% of the saturated hydrocarbons lighter than C_{24}. In all cases, the paraffins heavier than C_{26} display a marked odd-to-even preference, CPI_{21-29} falling in the range 2-3.5.

Isoprenoid hydrocarbons in 3-209, 3-324 and 3-534 are present trace amounts only ($\leq$ 10-20 ppb), whereas in 3-34, the pristane concentration is 665 ppb and the phytane concentration is 383 ppb. There is also evidence from ultra-violet spectroscopy for a greater aromatic hydrocarbon concentration in 3-34.

Hole 6

The hydrocarbon contents of the samples from 6A-15, 6-43 and 6-153 are 130, 240 and 120 ppb, respectively, and no pristane or phytane was identified. C_{29}, C_{31} and C_{33} (Fig. 5) show strong dominance, and account for $\sim$50% of the total hydrocarbons.

Hole 26

The concentration of saturated paraffins increased in samples from this site with depth, from 2.0 ppm in 26-100 to 2.4 ppm in 26-230 to 3.4 ppm in 26-478. Again, as in other samples , C_{29}, C_{31} and C_{33} predominate (Fig. 5) and the CPI for C_{25-31} is 3.3, 3.7 and 2.2 in the three samples, respectively. However, unlike the other samples, at least half the extracted hydrocarbons were branched. They showed a complex distribution over the entire hydrocarbon range (C_{15}-C_{33}) and no attempt was made to identify individual compounds.

Hole 27

The hydrocarbon content of the three samples at this site is very low (120 ppb for 27-143, 810 ppb for 27-237 and 320 ppb for 27-249). Again, there is a strong odd-to-even relationship with CPI_{21-31}= 2.2, 3.3 and 2.5 in the three samples, respectively. The long-chain hydrocarbons C_{25} to C_{33} predominate (Fig. 5).

Branched hydrocarbons are only present to 1/50 of the straight-chain components, and pristane is less abundant than phytane in all cases (pris./phyt. = 0.19, 0.43 and 0.43, respectively).

Perylene

Perylene ($C_{20}H_{12}$) was the only aromatic hydrocarbon identified quantitatively in this study. It was present in trace amounts in sample 3-34 of Hole 3 and in all three samples analysed in Hole 26 in quantities of 185 ppb for 26-100, 331 ppb for 26-242 and 161 ppb for 26-478. This compound was not detected in the other samples.

Fatty Acids and Alcohols

Hole 2

Sample 2-20 contained 660 ppb of hydrolyzable fatty acids (HFA) and sample 2-103 only 70 ppb. No free fatty acids (FFA) were detected. The dominant components in each case were n-C_{16}, C_{28} and C_{30}. No C_{16} unsaturated HFA was detected, and the ratio Δ-C_{18}/C_{18} was < 1 for both samples (Table 2). In both samples, HFA $> C_{20}$ were more abundant.

It was estimated that $<$ 70 ppb alcohols were present in each sample, even-numbered members, from C_{14} to C_{26} were detected.

Hole 3

Whereas no FFA were detected, HFA were identified in all the four samples from this site, the highest concentration (900 ppb) was in the top sample. The dominant acids were n-C_{16} and C_{18}. Saturated C_{16} was always more abundant than Δ-C_{16}

(Table 2), whereas, in 3-324 and 3-534 Δ-C_{18} was more abundant than C_{18}. These were the only two samples in the present study, which showed $\Delta\text{-}C_{18}/C_{18} > 1$.

Alcohols were detected in all four samples in concentrations equal to 25% of HFA. The identified alcohols were even-numbered compounds, the dominant alcohol was generally n-C_{20}, however, higher molecular weight compounds which were not identified constitute the bulk of this fraction. In sample 3-34, phytol was detected at the concentration level of 9 ppb, but no dihydrophytol could be measured.

Hole 6

Only traces of HFA ($<$ 50 ppb) were measured and no alcohols were detected in samples analyzed at this site.

Hole 26

This site is the only one in which FFA were identified. Concentrations in the three samples were 150 ppb in 26-100, 1,000 ppb in 26-230 and 140 ppb in 26-478 . The high concentration of FFA in 26-230 is mainly due to large amounts of even carbon fatty acids: C_{24}, C_{26}, C_{28} and C_{30}. The HFA, however, showed a gradual decrease from 2,790 ppb in 26-100 to 490 ppb in 26-478. In all cases, even carbon acids from C_{16} to C_{30} dominate over the odd carbon acids. Small quantities of Δ-C_{16} were present in the upper two samples, but could not be detected in 26-478 (Table 2). The ratio Δ-C_{18} to C_{18} was lower at this site than any other (Table 2).

The alcohol fractions consisted mainly of long straight-chained alcohols in the C_{20} to C_{30} range, with only trace amounts of C_{16} and C_{18}. The even carbon-numbered alcohols were dominant (CPI's $\approx$ 10),

with a maximum at C_{24}. Again, as in the case of the FFA, 26-230 contained the greatest concentration of alcohols (4.1 ppm, Table 3) greater than 26-100 (2.3 ppm) and 26-478 (0.6 ppm) and surprisingly exceeding the combined concentration of FFA and HFA (2.3 ppm). No isoprenoid or other branched alcohols could be identified in these sediments.

Hole 27

No FFA were found at this site. In both 27-143 and 27-249 C_{14} to C_{18} even-numbered straight-chained HFA dominate. The distribution in 27-237 is bimodal, with approximately equal quantities in the range C_{16}-C_{18} and C_{24}-C_{28}. Sample 27-143 has the greatest percentage content of unsaturated fatty acids. The $\Delta\ C_{18}/C_{18}$ is 0.78 (Table 2). This ratio also describes the distribution of Δ-C_{18}/C_{18} in 27-237. However, in this sample the HFA concentration (2.5 ppm) is an order of magnitude greater than that in 27-249 and nearly two orders of magnitude greater than the HFA in 27-143.

Apart from sample 27-237 where the alcohol concentration was 1.4 ppm, the other two samples had $<$ 100 ppb alcohol (Table 3). The straight-chain compounds extracted are dominated by C_{22}-C_{28} even-numbered moieties.

Chlorins and Porphyrins

In samples from all sites, except Hole 3, chlorins and porphyrins were either absent (Hole 6) or mutually exculsive (Table 3). In Hole 2, only porphyrins were present (19 and 7 ppb), and from the spectrum shown in Fig. 6, it was deduced by comparison with spectra from known porphyrins (Hodgson et al. 1968) that the compound is a vanadyl porphyrin. In Holes 26

and 27, it was not possible to determine the porphyrin type with any confidence. The soret absorption peaks for porphyrin from samples 26-478, 27-143 and 27-249 lay between 395 and 408 mμ. However, the peaks were not very sharp due to superposition of other absorption peaks, probably aromatic hydrocarbons, which co-eluted with the porphyrins and chlorins. The non-soret peaks for samples from Hole 27 were very shallow, whereas in sample 26-478, two non-soret peaks at 555 and 585 mμ were measured, indicating a possible mixture of at least two specific porphyrins.

Best resolution was obtained in samples from Hole 3, especially 3-34. Here, it was possible to separate two compounds with absorption properties similar to Ni-porphyrin (392, 512 and 549 mμ) and the other with an absorption spectrum (405-407, 530, 570 mμ) similar to V-porphyrin (see Fig. 5). Two porphyrins with similar absorption characteristics were also separated from sample 3-534, indicating a V-porphyrin and Ni-porphyrin mixture. In this sample, the ratio of V/Ni complex = 13, whereas in 3-34, V/Ni complex = 4.

When present, the chlorin content decreases with depth. They can be recognized by their absorption peak at 663-665 mμ, as shown for 3-34 in Fig. 6. However, the concentration of chlorins was found to be very great in sample 26-100 (568 ppb) and it was possible to determine other non-soret peaks at 668, 610, 535 and 505 mμ (Fig. 6). These absorption peaks were also measured by Baker (1970) in Hole 26 from a sample at 120 m depth and interpreted by him as typical of pheophytin. Hence, the chlorins in sample 26-100 and 26-230 still contain the phytol side chain.

Amino Acids and Racemization

Because of the relatively high concentration of organic carbon and the relatively good preservation of the chlorins and fatty acids in the sediment at site 26, the three samples obtained from Hole 26 were analysed for hydrolyzable amino acids (a.a.). The concentration was greatest in sample 26-100 (500 n-moles/g) and decreased with depth (390 n-moles/g for 26-230 and 75 n-moles/g for 26-478) The acidic a.a. (e.g., glutamic and aspartic) decreased most rapidly followed by hydroxy-amino acids. The neutral acids were more stable. The general pattern of degradation shown in Fig. 8 confirms studies by Brown et al. (1972) on sediment from a fjord. In that study, the basic a.a. were found to be the least degraded.

Although degradation in the sediment column probably results from microbiological alteration (i.e., deamination), amino acids have a defined lifetime even under sterile conditions, which may be predictable (Abelson 1963). Racemization of the enantiomers is another measure of alteration (Hare and Abelson, 1967; Bada et al. 1970; Wehmiller and Hare, 1971), and was shown to occur in a.a. from reducing sediment of a fjord by Kvenvolden et al. (1970). The data in Table 4 shows that in sample 26-100, serine, alanine and glutamic acid already show very significant racemization. The greatest change is seen in sample 26-478, where alanine shows 47% conversion to the D-form. At this depth, phenylalanine, leucine, valine and proline also show significant conversion.

Using the method of Bada et al. (1970) for calculating sediment age by the rate of epimerization of L-isoleucine to D-alloisoleucine (estimated by plotting ln [1 + alloisoleucine/isoleucine] versus depth of burial), one obtains an age for 26-478 = 1.2×10^6 years. This agrees with the general stratigraphic age of the sediment (Upper-Middle Pleistocene; Bader et al. 1970). However, many pitfalls exist in the application of this method for dating sediment (Wehmiller and Hare, 1971). It can further be seen from Table 4 that racemization does not progress smoothly down the core, as the sediment from 26-230 shows less L-D conversion for serine and alanine than is found in the overlying sample 26-100. In part, this is due to source material (whether planktonic or terrigenous) and partly due to the conditions of accumulation. Furthermore, new a.a. are being generated in the column. For example, the non-protein amino acids β-alanine and γ-aminobutyric acid are present in higher concentration at depth 230 m than at 100 m. These acids could conceivably have been introduced at the surface by diverse plant remains. However, the percent of β - alanine and γ-aminobutyric relative to the total acids increases dramatically down the sediment column (13% at 100 m, 44% at 230 m and 70% at 478 m). They were probably derived by decarboxylation from other amino acids such as aspartic and glutamic acids. Eventually, death of the indigenous microflora will also release new L-amino acids into the sediment.

Diagenetic Pathway of Carbon Compounds

It is apparent from results in Figure 8 and Table 5 that the lipid-associated compounds and protein-derived amino acids

generally decrease in the sediment column. Their combined contribution to the organic carbon in the sediment is less than 1% (Tables 5 and 6). In the organic extractable fraction (Table 5) the hydrocarbons become the most abundant component, except for very young sediment (as in Hole 26) which has been rapidly deposited. Here, free fatty acids and chlorins can also be detected, however, the total content of this extractable fraction was found to be less than 0.1% of the total organic carbon in that sample. Only in sample 2-20, where 17 ppm n-alkyl hydrocarbon was extracted was there an increase above this relative concentration.

It is therefore obvious that biogenic-derived organic matter rapidly transforms to stable configurations or else degrades. It is known of course, that carbon compounds are degraded in the sediment, in part because of a small decrease with depth of burial, but also because of excess CO_2 liberated into the interstitial water (Presley and Kaplan 1968) and release of ammonia, methane and hydrogen sulfide (Nissenbaum et al. 1972 b). Previous studies on very young near-shore reducing sediment (Brown et al. 1972) have shown that the humic acid fraction (0.2N NaOH-soluble and acid-insoluble) may contain 40% of the total carbon and another 30% is present in the fulvic acid fraction. The fulvic acid content decreases with depth of burial, and in sediment nearly 10,000 years old, it is ~9%. In the present study (Table 6) it can be seen that the humic acid fraction represents from 27-43% of identifiable carbon. The fulvic acid content analysed varied from 5-18%, but because of difficulty in purification (during removal of salt) reproducible values could not be obtained and are therefore not recorded in Table 6. The non-

extractable fraction (Kerogen) represents only 10-14% of the total carbon. Therefore, about 50% or more of the carbon has not been accounted for. To test where this loss occurred, total organic carbon remaining in the sediment was measured by combustion on a small aliquot, after each extraction. It was found that the carbon was lost, in part, during acid hydrolysis but mostly during alkali extraction. Cleavage of small soluble molecules from the complex humic acid has occurred. Those with a molecular weight of several thousands, the fulvic acids, may amount to 10 or 20% of these unaccountable compounds. Those which can pass through a dialysis membrane (M.W. $<$ 2,000) but resemble components of the fulvic and humic acids are lost. These are probably peptide, carbohydrate and aromatic constituents with hydrophyllic groups.

DISCUSSION

Several conclusions have emerged from the study. First, it is apparent that burial under conditions where heat flow is insufficient to raise the temperature beyond 30 or 40°C, causes disappearance (removal) of most of the labile biochemical components originally deposited in the sediment. There is no evidence that petroleum can form under these conditions, although small quantities of hydrocarbons (in the ppm range) may be generated (as appears to be happening in Hole 26).

The major sink for the non-decomposed buried carbon compounds in unlithified sediment, is humic acid and to a lesser extent, kerogen. Some compounds are strongly complexed and will precipitate with the humic acid polymer. Others will be solubilized on acid treatment of the alkaline humic acid solution (the fulvic

acids), whereas less complexed groups will become soluble in distilled water or weak brine. It is probable that continued dehydration during lithification would convert proportionately more of the humic acid fraction to kerogen, although we have no proof for this, in the present study.

The environment of deposition and accumulation is most important for the preservation of organic matter. Slow deposition in deep water of pelagic sediment, or exposure to surface currents after rapid deposition (by turbidity currents) could lead to destruction of most of the labile molecules prior to effective burial. This has probably occurred at site 6 and 6A and to a lesser extent, site 2. Oxidation would rapidly remove free fatty acids, carotenoids (which could not be identified in this study) and chlorophyll derivatives.

Porphyrins would form under mild oxidizing conditions, preceeded by loss of the phytyl group. The presence of porphyrins in the relatively young sediment of Hole 2 and Hole 3 may suggest an external source rather than generation in situ. This is particularly true for sample 3-34, which contains both chlorins and porphyrins.

Although this study and that by Koons (1970), do not show a clear relationship between the hydrocarbons identified from sediment of Holes 2 and 3 and the petroleum removed from Hole 2 above Challenger Knoll, there may be horizontal or vertical migration from various reservoirs. The hydrocarbon content in sample 2-20 is 4 times greater than in 2-103, and that of sample 3-34, 9 times greater than the other samples in Hole 3, which argues against a simple upward migration. However, the n-alkyl

fractions here are greater than in most other unconsolidated Quaternary sediments. These samples differ from young sediments in two other respects, the presence of porphyrins, as stated earlier, and the presence of aromatic hydrocarbons.

Of particular interest is the sedimentological evidence for detrital minerals in many of the organic-rich cores. Evidence was also produced for plant debris in Hole 26 and turbidity current deposits associated with Hole 27. Both of these sites are very large distances from land. The geochemical evidence obtained here indicates that land-derived organic matter may be more common than previously assumed.

Data shown in Tables 1 and 6 for C^{13}/C^{12} ratios indicate that samples 3-34, 26-230 and 27-237 have δC^{13} ratios typical of land plants (see Nissenbaum and Kaplan 1972). The light isotopic values for the total organic carbon in samples 26-230 and 27-237 are also reflected in the humic acids and kerogen (Table 6). Carbon isotope values for plankton-derived organic matter is more normally -18 to -22‰. It has been suggested that organic matter in marine sediment with light isotopic ratios (approaching -30‰ PDB) may be due to growth in cool water (Rogers and Koons 1969), however, this interpretation is speculative and may only be applicable in very specific environments (Sackett and Rankin 1970; Plucker 1970).

The presence of long-chain hydrocarbons (C_{25}-C_{32}) and also long-chain alcohols suggests that plant waxes may have been the contributing source. In fact, the n-alkanes of all the samples analyzed in this study had high CPI's (2.0 to 4.6), which indicates terrigenous contribution. A particularly interesting

index is perylene $C_{20}H_{12}$. Aizenshtat (1972) has shown that this molecule probably originates from soil as the precursor 4,9-dihydroxyperylene-3,10-quinone. This compound is highly susceptible to oxidation, and therefore must be rapidly deposited under reducing conditions. Marine sediment primarily receiving planktonic organic matter does not contain perylene. Near-shore sediment deposited under oxidizing conditions also do not contain perylene (Aizenshtat 1972).

SUMMARY

The results of the study indicate that degradation and diagenesis of organic matter occurs in steps. The important factors to be considered are the following:

1. Environment of deposition. Initially, this factor is of overriding importance. Irrespective of the quantity and nature of organic matter being deposited under natural conditions, if the path through which it deposits permits exposure to oxygen, a great majority of the organic matter will be decomposed. This generally occurs during settling through a long water column to abyssal or haedal depths. Furthermore, pelagic sedimentation in the bathyal environment is generally very slow ($<$ 1 cm/1,000 yrs.) and permits deposited material to be degraded once it has settled. Site 6 described in the present study falls into this category of environment.

2. Source of material. If deposition is relatively rapid and the environment of deposition allows organic matter to be

buried, its alteration path will depend on the nature of the initial organic matter. Land-derived higher plant material containing lignins and waxes are probably not degraded to CO_2 as effectively as plankton-derived organic matter. The terrigenous origin of starting material can be recognized by several indices described in this paper; (i) δC^{13} values $< -26‰$, (ii) long-chain alcohols and hydrocarbons ($> C_{27}$), (iii) CPI > 2 in the hydrocarbons and (iv) presence of the aromatic hydrocarbon perylene.

3. Environment of accumulation. Deposition on the sea floor by means of turbidity or density currents may be rapid. However, these may occur at infrequent periods, so that re-working of the surface sediment by bottom currents and benthonic organisms may prevent the preservation of "delicate" components such as unsaturated fatty acids, chlorins or the quinoid precursors of perylene. In such an event, evidence for source origins based on presence or absence of ephemeral compounds may be misleading. This explanation can probably be applied to the organic matter of site 26.

4. Alteration pathway. The two most pronounced patterns are: (a) degradation of recognizable biological polymers (e.g., cellulose, proteins) and (b) formation of new condensed components enriched in hydrophylic groups and probably aromatic compounds. Carotenoids disappear early, chlorophyll alters to phaeochlorins which may almost disappear, extractable amino acids amount to $<$ 1% of the total organic reservoir. The humic and fulvic acid components assume 50% of the total extractable organic material. In fact, they may represent 80%+ of the organic matter,

but are hydrolyzed during alkaline extraction, at which time the smaller more soluble fragments are lost. Many unaltered or partially altered compounds may be either complexed or strongly adsorbed to these high-molecular weight components. Kerogen appears to constitute <20% of the organic matter in the unlithified sediment. New molecules, such as perylene or porphyrins, may appear.

5. Alteration during lithification. Diagenesis during lithification will involve dehydration and possibly heating. The humic acid complexes may begin to degrade, releasing their constitutive compounds. The remainder may become less soluble and will then be termed kerogen. The alteration from humic acid to kerogen is not understood and few examples have yet been studied in sediment passing the phase transition from mud to shale.

AKNOWLEDGMENTS

We wish to thank Keith Kvenvolden and Etta Peterson for identification and quantitative determination of the amino acids. This study was supported by a grant from NASA, NGR 05-007-221.

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TABLE 1: Description of Samples

Location	Water Depth (m)	Sample Hole - Depth (m)	JOIDES Designation[1]	Age	Description	% Org.C.	δC^{13} (‰)
23° 27.3'N	3572	2 - 20	1-2 (0- 65)	Pleist.	Calcareous mud	0.45	-23.4
92° 35.2'W		2 - 103	4-1 (30- 90)	Pliocene	Cocc. ooze, H_2S	0.38	-22.8
23° 01.8'N	3747	3 - 34	1-2 (0- 72)	Pleist.	Calc. silt, clay	1.11	-26.6
92° 02.6'W		3 - 209	4-1 (33-100)	Pleist.	Cocc. ooze, clay	0.82	-21.7
		3 - 324	5-2 (102-150)	Pliocene	Cocc. ooze, clay	0.47	-22.1
		3 - 534	9-2 (18- 60)	Miocene	Grayish-green silty clay	0.47	-22.2
30° 50.39'N	5125	6A - 15	1-2 (10- 72)	Pleist.	Brown clay	0.13	-24.0
67° 38.86'W		6 - 43	1-1 (90-150)	Pliocene	Brown clay	0.16	-25.4
		6 - 153	2-2 (0- 70)	Eocene	Gray-green clay	0.09	-25.7
10° 53.55'N	5168	26 - 100	1-3 (81-130)	Pleist.	Gray silty clay	0.87	-25.3
44° 02.57'W		26 - 230	3-2 (100-150)	Pleist.	Olive-gray clay-claystone	1.00	-27.0
		26 - 478	5-1 (89-131)	Pleist.	Dk. olive gray silty claystone	0.51	-25.2
15° 51.39'N	5258	27 - 143	2-2 (95-150)	Miocene	Lt. olive-gray clay	0.27	-25.0
56° 52.76'W		27 - 237	3-2 (0- 64)	Miocene	Stiff green-gray clay	0.58	-26.8
		27 - 249	4-3 (84-150)	Miocene	Green-yellow mottled clay	0.18	-24.5

[1] Core-section (interval)

TABLE 2. Hydrolyzable Fatty Acids

Hole-depth (m)	Total μg/100g[±]	% > C_{20}	% Unsaturated	$\Delta 16/16$	$\Delta 18/18$
2 - 20	60	67	5	--	0.69
2 - 103	7	71	4	--	0.75
3 - 34	90	15	14	0.12	0.60
3 - 209	29	11	14	0.08	0.78
3 - 324	36	8	30	0.10	2.64
3 - 534	41	15	23	0.13	1.35
26 - 100	279	83	3	0.14	0.53
26 - 230	130	69	4	0.07	0.40
26 - 478	49	63	3	--	0.27
27 - 143	3	--	22	0.17	0.78
27 - 237	250	53	8	0.08	0.77
27 - 249	28	15	13	0.12	0.52

[±] Fatty acids identified range in carbon number from C_{14} to C_{30} with the exception of Hole 3 which ranges from C_{14} to C_{24}.

Table 3

Concentration of identified compounds from benzene-methanol extract (μg/100 g)

Hole	Depth (m)	n-Alkanes	Perylene	FFA	HFA	Alc.	Chlorins	Porphyrins
2	20	1700	a.	a.	60	~7	a.	1.9
2	103	403	a.	a.	7	~7	a.	0.7
3	34	930	t.	a.	90	23	8.1	7.1
3	209	59	a.	a.	29	5	4.9	0.9
3	324	114	a.	a.	36	3	0.8	1.0
3	534	102	a.	a.	41	9	a.	2.3
6A	15	13	a.	a.	< 5	a.	a.	a.
6	43	24	a.	a.	< 5	a.	a.	a.
6	153	12	a.	a.	< 5	a.	a.	a.
26	100	191	18.5	15	279	233	56.8	a.
26	230	242	33.1	100	130	407	14.6	a.
26	478	344	16.1	14	49	60	a.	1.8
27	143	12	a.	a.	3	9	a.	0.2
27	237	81	a.	a.	250	136	1.2	a.
27	249	32	a.	a.	28	4	a.	0.8

a. = none detected

t. = trace

TABLE 4: %D enantiomer of amino acids from Hole 26

Amino Acid	Sample depth (m)			
	100	230	478	Δ**
Ala	21.0	19.0	47.1	26.1
Val	6.2*	12.6*	22.7	16.5
Aileu	4.5*	6.9*	14.2*	9.7
Leu	7.8	15.0	28.6	20.8
Pro	11.9*	14.0*	24.8*	12.9
Ser	29.9	10.5	35.2*	5.3
Phe	10.9	15.3	32.2	21.3
Glu	19.2*	24.3*	34.8*	15.6

Results are an average of data from two capillary columns coated with different liquid phases--UCON 75H-90,000 and XE-60. (Kvenvolden et al., 1970)

* Results from only one column.
** 478 minus 100

Table 5

ORGANIC CONSTITUENTS IDENTIFIED IN JOIDES SAMPLES

% of total Org. C X 10^2

Hole	Depth (m)	n-Alkanes	Perylene	FFA	HFA	Alc.	Chlorins	Porphyrins
2	20	32.16	a.	a.	1.04	~0.13	a.	0.03
2	103	9.03	a.	a.	0.14	~0.15	a.	0.01
3	34	7.14	a.	a.	0.63	0.17	0.05	0.05
3	209	0.61	a.	a.	0.28	0.05	0.04	0.01
3	324	2.06	a.	a.	0.60	0.05	0.01	0.02
3	534	1.85	a.	a.	0.68	0.16	a.	0.04
6A	15	0.85	a.	a.	< 0.30	a.	a.	a.
6	43	1.28	a	a.	< 0.30	a.	a.	a.
6	153	1.14	a.	a.	< 0.30	a.	a.	a.
26	100	1.87	0.20	0.13	2.50	2.19	0.49	a.
26	230	2.06	0.32	0.78	1.01	3.32	0.11	a.
26	478	5.38	0.30	0.21	0.75	0.96	a.	0.03
27	143	0.38	a.	a.	0.09	0.27	a.	0.01
27	237	1.19	a.	a.	3.35	1.91	0.02	a.
27	249	1.51	a.	a.	1.21	0.18	a.	0.03

a. = none detected

TABLE 6: Organic Groups Identified in Hole 26

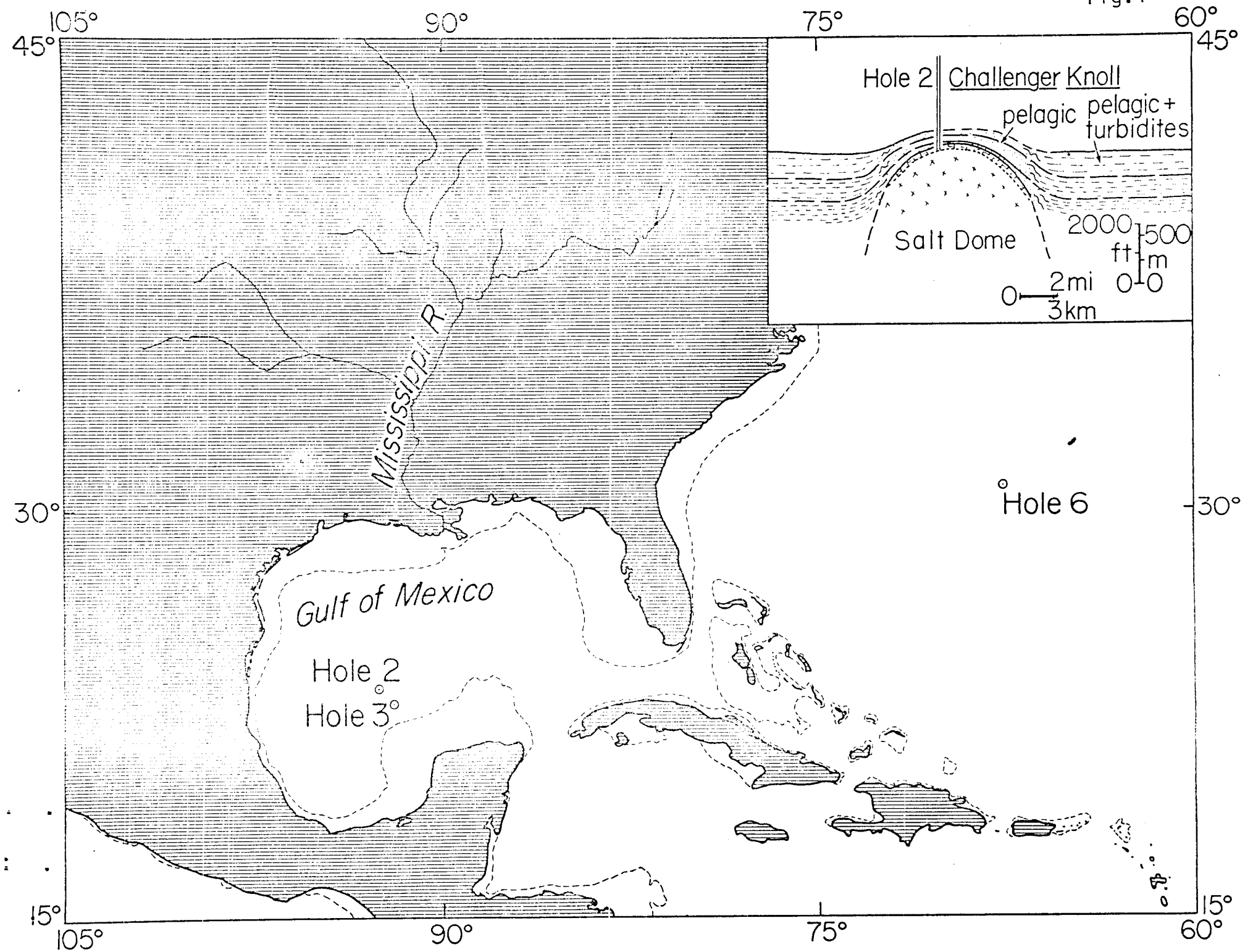
% of total Org. C and δC^{13}

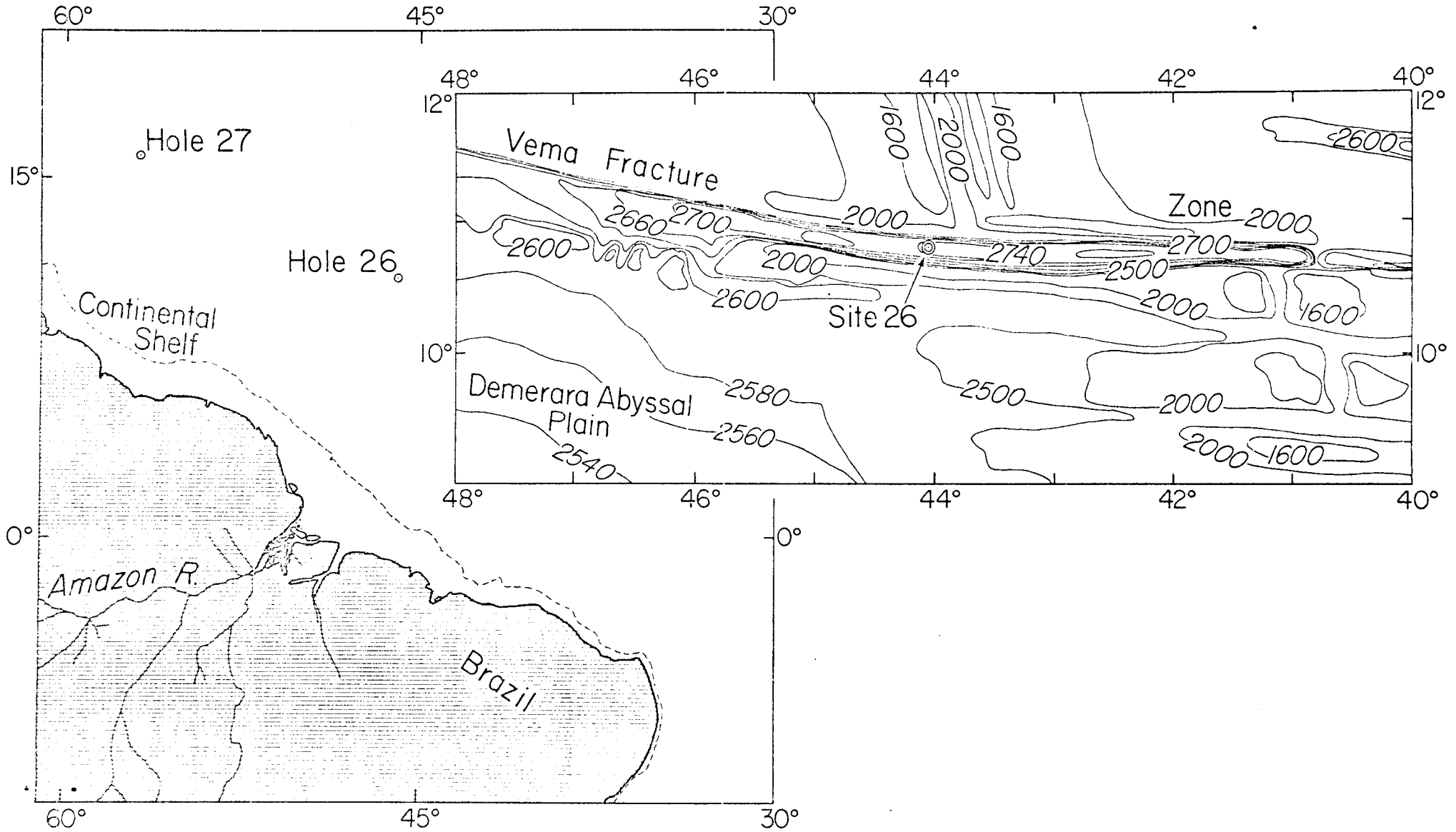
Sample (δC^{13})*	Benzene soluble	Amino acids	Humic acics (δC^{13})	Kerogen (δC^{13})	Remainder
26-100 (-25.3)	< 0.1	0.3	28 (-24.7)	11 (-25.1)	61
26-230 (-27.0)	< 0.1	0.2	43 (-25.8)	10 (-26.6)	47
26-478 (-25.2)	<0.1	0.1	27 (-24.4)	14 (-25.6)	59

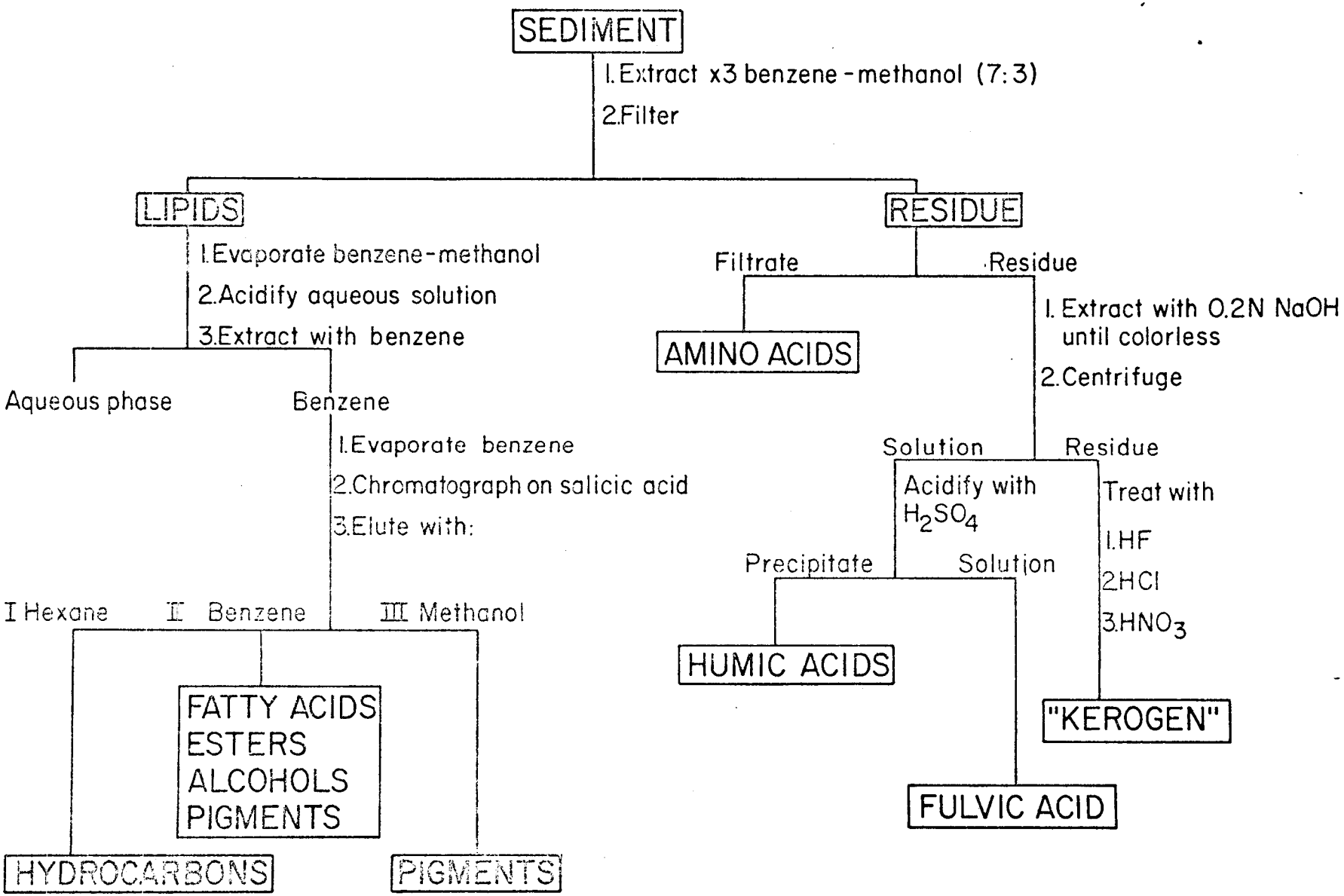
* °/oo relative to PDB standard

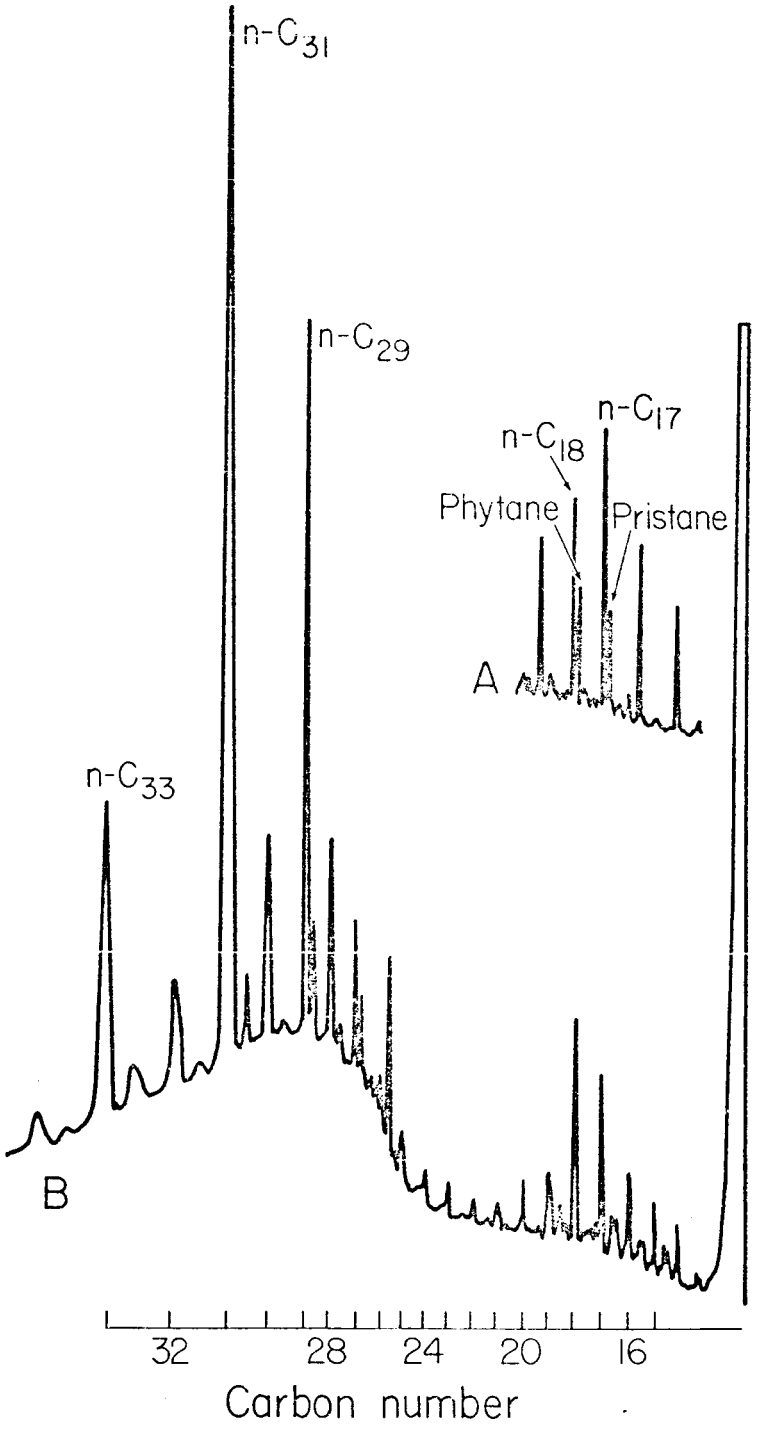
LIST OF FIGURES

Fig. 1


105°
90°
75°
60°
45°
30°
15°
Hole 2
Challenger Knoll
pelagic
pelagic + turbidites
Salt Dome
2000
1500
ft
m
0
2mi
3km
0
Mississippi R.
Hole 6
Gulf of Mexico
Hole 2
Hole 3


60°
45°
30°
48°
46°
44°
42°
40°
12°
15°
10°
0°
Hole 27
Hole 26
Vema Fracture Zone
Site 26
Continental Shelf
Demerara Abyssal Plain
Amazon R.
Brazil
1600
2000
2600
2660
2700
2740
2500
2580
2560
2540


SEDIMENT
1. Extract x3 benzene - methanol (7:3)
2. Filter
LIPIDS
1. Evaporate benzene-methanol
2. Acidify aqueous solution
3. Extract with benzene
Aqueous phase
Benzene
1. Evaporate benzene
2. Chromatograph on salicic acid
3. Elute with:
I Hexane
II Benzene
III Methanol
FATTY ACIDS
ESTERS
ALCOHOLS
PIGMENTS
HYDROCARBONS
PIGMENTS
RESIDUE
Filtrate
Residue
AMINO ACIDS
1. Extract with 0.2N NaOH until colorless
2. Centrifuge
Solution
Residue
Acidify with H_2SO_4
Treat with
1. HF
2. HCl
3. HNO_3
Precipitate
Solution
HUMIC ACIDS
"KEROGEN"
FULVIC ACID


n-C31
n-C29
n-C17
n-C18
Phytane
Pristane
A
n-C33
B
32
28
24
20
16
Carbon number

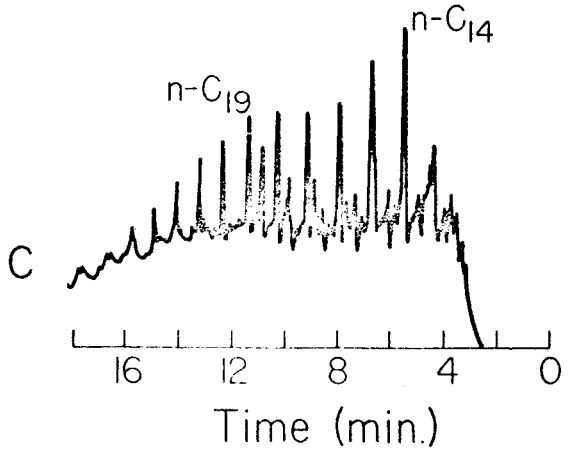

n-C14
n-C19
C
16
12
8
4
0
Time (min.)

Fig. 5

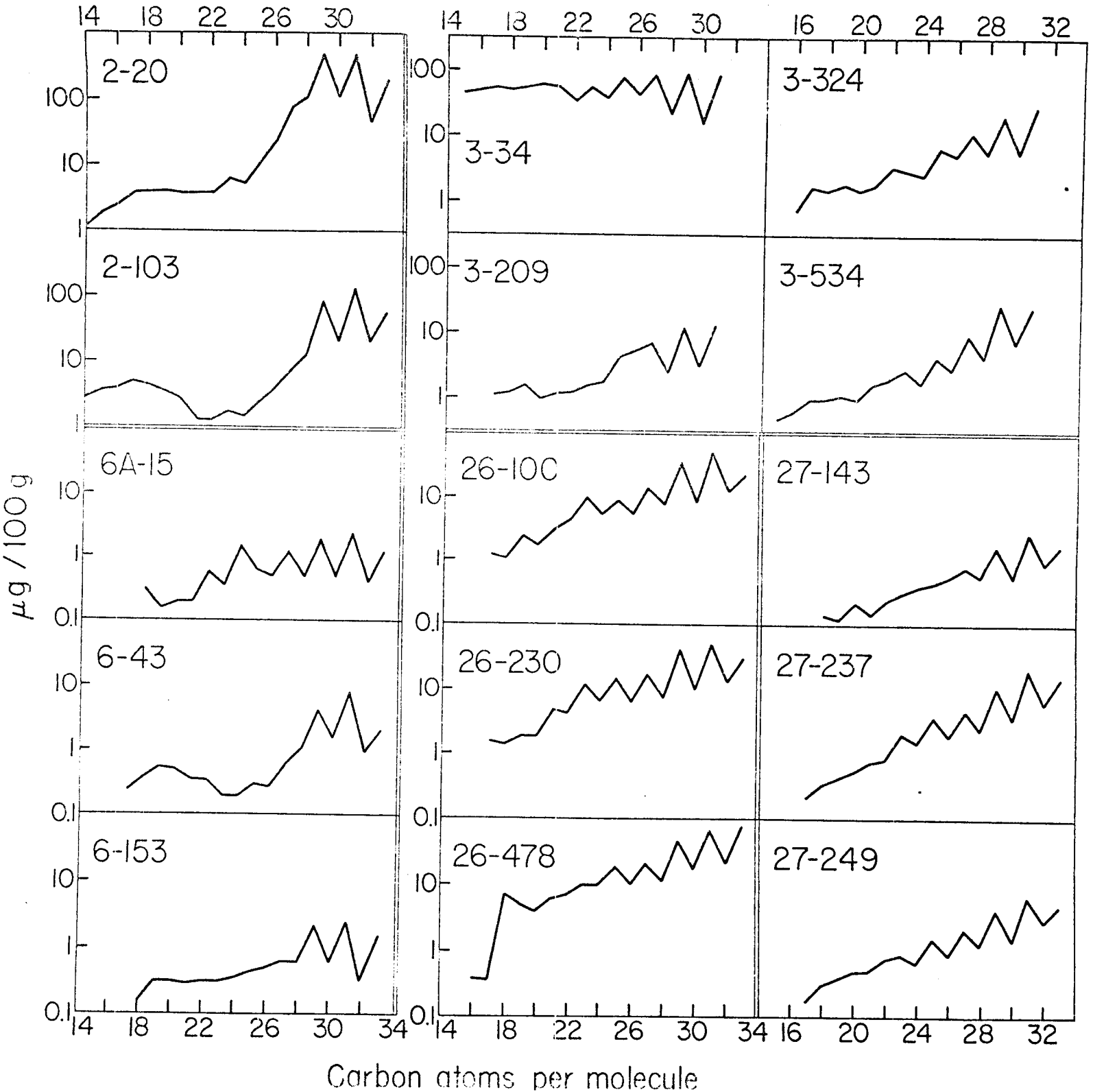
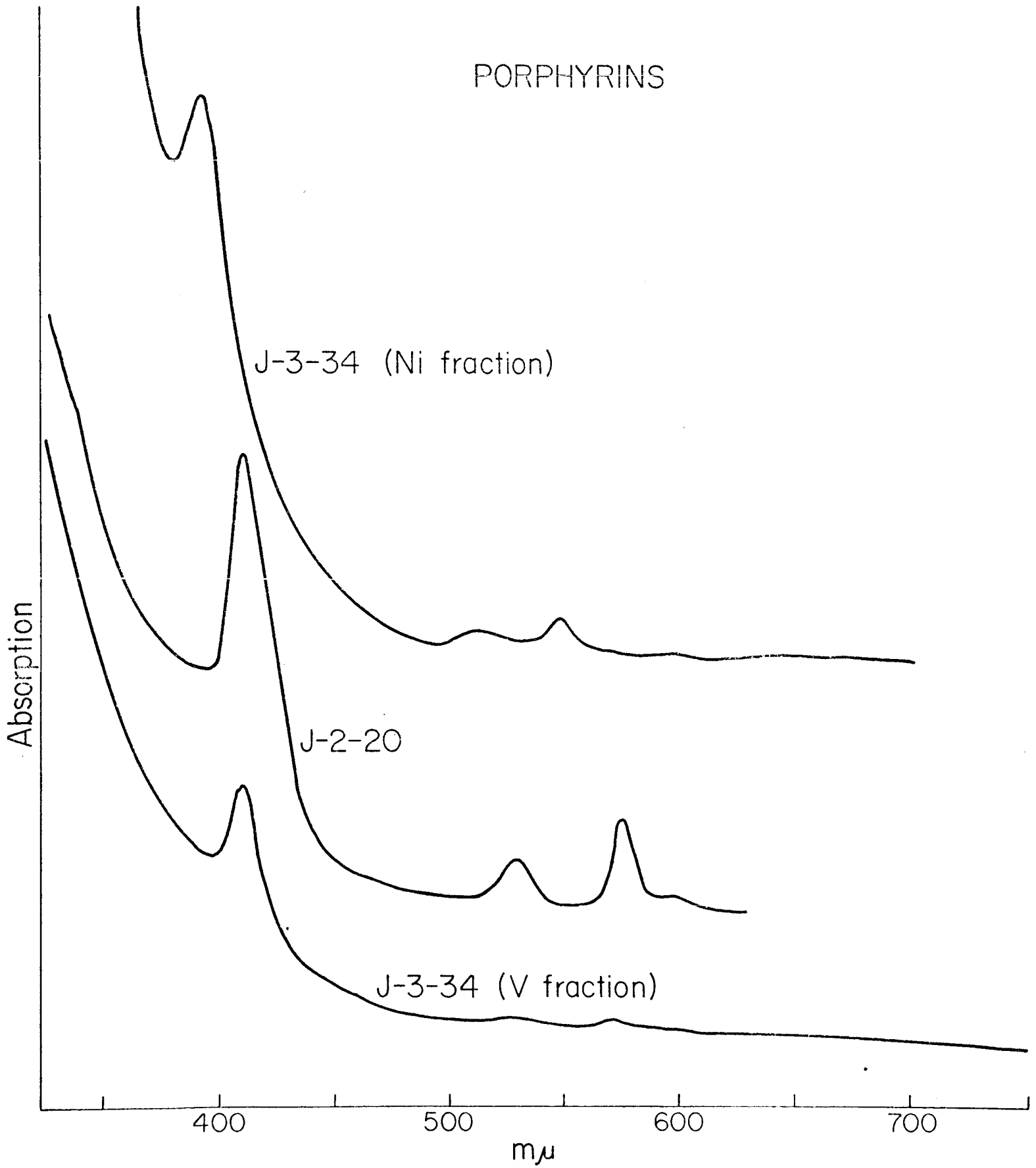
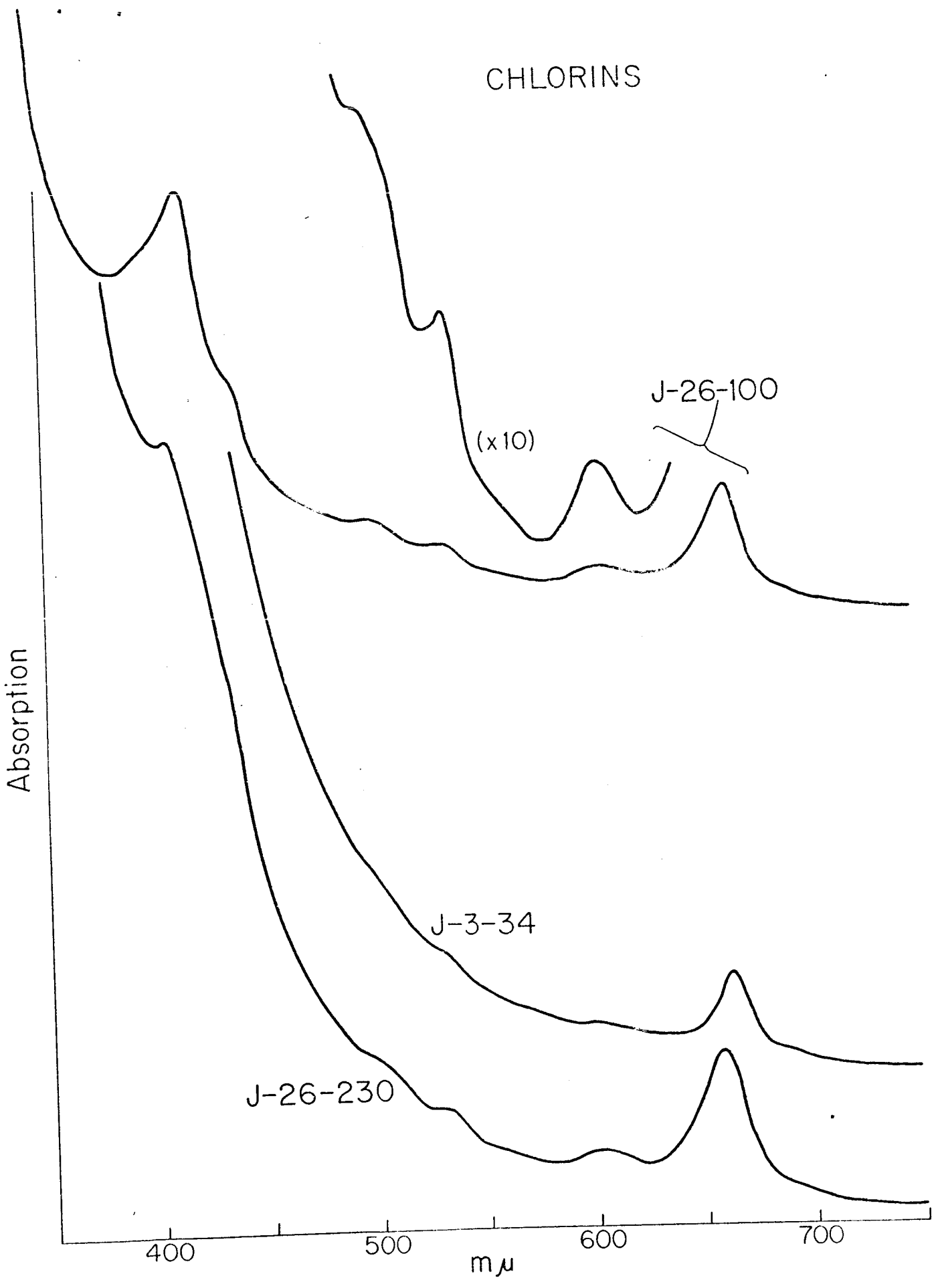
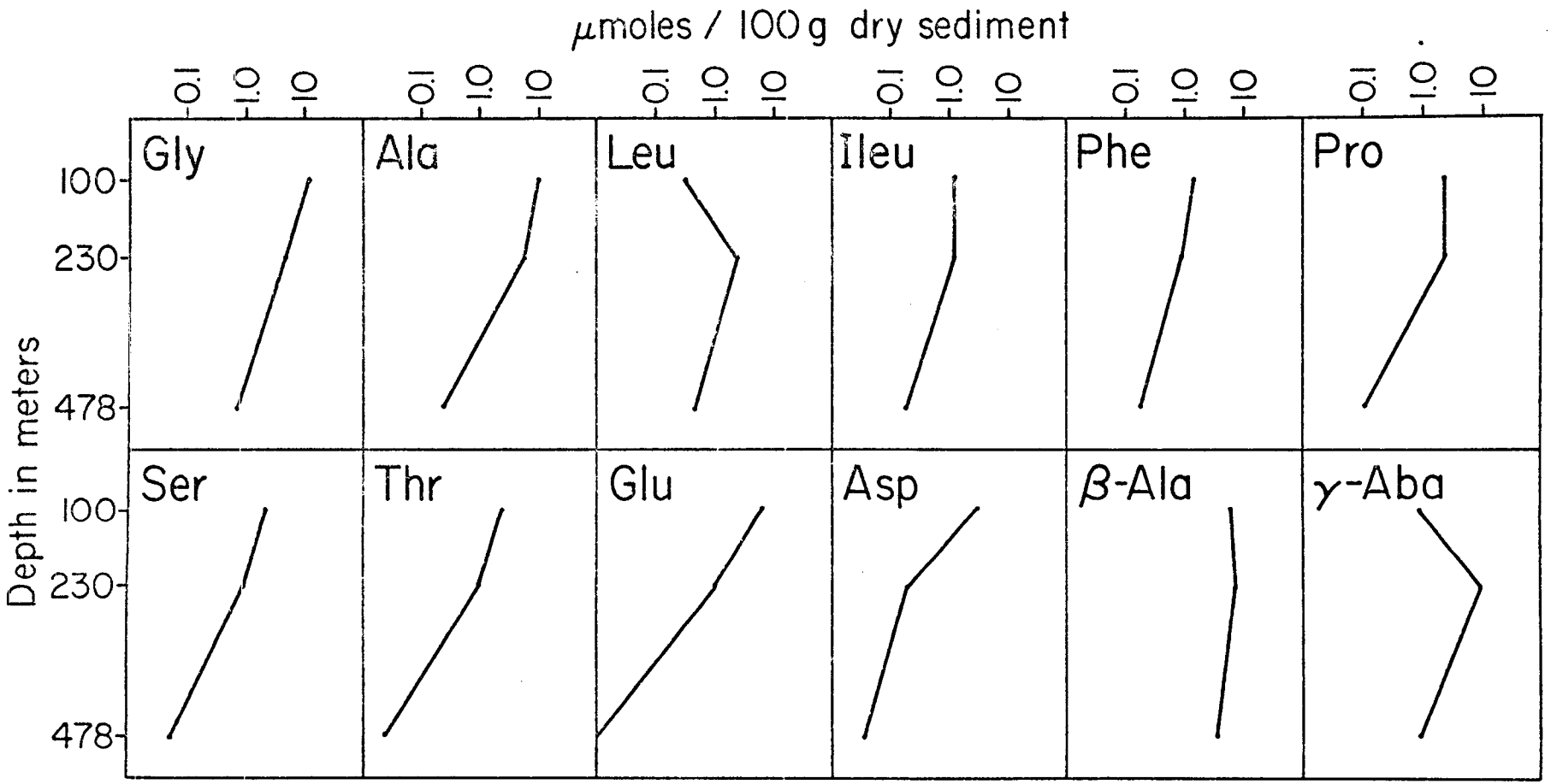

2-20
2-103
3-34
3-209
3-324
3-534
6A-15
6-43
6-153
26-10C
26-230
26-478
27-143
27-237
27-249
μg / 100 g
Carbon atoms per molecule

Fig. 6


PORPHYRINS
J-3-34 (Ni fraction)
J-2-20
J-3-34 (V fraction)
Absorption
400
500
600
700
mμ


CHLORINS
J-26-100
(x10)
J-3-34
J-26-230
Absorption
400
500
600
700
mμ


μmoles / 100 g dry sediment
0.1
1.0
10
Gly
Ala
Leu
Ileu
Phe
Pro
Ser
Thr
Glu
Asp
β-Ala
γ-Aba
Depth in meters
100
230
478

GROWTH PATTERN AND C^{13}/C^{12} ISOTOPE FRACTIONATION OF *CYANIDIUM CALDARIUM* AND HOT SPRING ALGAL MATS

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Photosynthetic activity of Cyanidium caldarium grown under pure CO_2 was recently reported (Sechbach et al. 1970; Sechbach et al. 1971). We now provide additional data for this thermophilic-acidophilic alga concerning its growth and C^{13}/C^{12} isotope fractionation characteristics. This hot spring alga was grown in a CO_2 atmosphere at different temperatures for various periods of time while monitoring the pH. Controls cultured in laboratory air were grown in parallel.

Data for the C isotope compositions were determined by combustion of the cells in a vacuum system using the modified technique described by Craig (1953). The CO_2 collected was transferred to a 6-inch, 60° radius, dual-collecting mass spectrometer manufactured by Nuclide, Inc. (Pennsylvania, U.S.A.), and measured against the UCLA calcite standard. All results are quoted relative to PDB. The δ value indicate the difference in per mil of the C^{13}/C^{12} ratio of the sample relative to a standard, and is defined as:

$$\delta(‰) = \frac{C^{13}/C^{12}\ \text{sample} - C^{13}/C^{12}\ \text{standard}}{C^{13}/C^{12}\ \text{standard}} \times 10^3$$

δC^{13} for air CO_2 was taken as -7‰, whereas for tank CO_2 it was measured as -34 ‰ in experiments carried out at UCLA and -30 ‰ in CO_2 used at the Hebrew University.

Measurements on C^{13}/C^{12} isotope ratios in photosynthetic systems, indicate that most higher plants (including all lower vascular plants and all gymnosperms except Welwitchia) yield δC^{13} values of -24 to -34 ‰ (Smith and Epstein 1971). Algae,

lichens and nanoplankton generally yield values -12 to -20 ‰ (Park and Epstein 1960). One possible explanation suggested for the difference, is that the algae are metabolizing bicarbonate, which is about 7 or 8 ‰ more enriched in C^{13} than atmospheric or dissolved CO_2. The increase in solubility of CO_2 with decrease in temperature was used to explain high enrichment in C^{12} ($\delta C^{13} \approx -26$ ‰) in algae grown at low temperatures (10°C) in the laboratory (Deuser et al. 1968; Degens et al. 1968a) and in ocean plankton hauls obtained in high latitudes (Degens et al. 1968b). This effect is of potential ecological significance, as a means of determining environment of growth by studying organic matter in sediment. C. caldarium was especially chosen for this study as it grows at low pH and, therefore, must only fix dissolved CO_2.

RESULTS AND DISCUSSION

The cultures were grown in agitated mineral media and aspirated with CO_2 or air as previously described (Sechbach et al. 1971). Typical growth curves of Cyanidium caldarium cells grown at room temperature (26°) and at elevated temperature (45°) are presented in Figure 1A and B, respectively. Growth value is expressed as the optical density of the suspension at 580 nm which also gives an estimate of the volume (ml/l) of packed cells. It is clear from the growth of Cyanidium at room temperature (Fig. 1A) that cultures grown on pure CO_2 have a lag period of adaptation of nearly two weeks and then show a higher rate of cellular increase, whereas aerated cultures show a growth response within one or two days. When Cyanidium is incubated under CO_2 at 45° (Fig. 1B), visible growth is observed within 24-48 hours and the growth rate is higher than in the aerated control, in contrast to results

obtained with CO_2 at room temperature (Fig. 1A). The growth curves shown in Figs. 1A and 1B were compiled from three different experiments. An additional characteristic of this organism, is the decrease in pH (Fig. 2) in the liquid growth media to $\approx$ 2 or lower.

Table 1 illustrates the temperature influence of carbon isotope composition in Cyanidium grown in CO_2 or in air. An increase of temperature from 25° or 26° C to 45° or 50°C in the air-grown cells does not result in any significant change in the cellular isotopic ratio of the cells ($\Delta\delta C^{13} = -11.7‰$ at 25°C and -13.5‰ at 40-50°C). CO_2-grown cultures consistently show a greater enrichment in the light isotope (decrease in δC^{13}) at the higher temperature than air-grown cells (Ave. $\Delta\delta C^{13} \approx -18.4‰$ for range 40°-50°). Two cultures grown on CO_2 at 25° and 26°C have $\Delta\delta C^{13}$ values -5‰ and -11‰, respectively.

These results are in contrast to the normally-accepted biogenic isotope effect, which predicts that the kinetically-controlled process should show greater fractionation at lower metabolic rates (lower temperatures). From data presented in Table 1, attention should be focused on two values for CO_2-grown cells at 26°C in Experiment 1. When cells were still in their early growth stage (14 days old) the analyzed value was -11‰, whereas, after 41 days of growth, the culture yielded a measured $\Delta\delta C^{13}$ value of -19‰.

Additional information on the stable C isotope distribution was obtained from lipid fractions extracted from anaerobically (CO_2) and aerated grown Cyanidium cells. Table 2 demonstrates that C^{13}/C^{12} ratios in the lipid fraction of CO_2-grown cells

is similar to the ratio of unextracted cell components, whereas in air-grown cells, the light isotope is enriched preferentially in the lipid carbon as previously observed in plankton . The lipid fractions were obtained from ca. 100 mg lyophilized algae powder which was extracted by refluxing over a boiling water bath with 50 ml of methanol:chloroform:benzene (1:2:2) for 2.5 hours. The extract was centrifuged for 30 minutes and the lipid supernatant was removed from the residue, both fractions were dried prior to the C^{13} determination.

Increase in temperature and decrease in the nutrient pH lowers the solubility of CO_2, which is 1.7 times higher for water at 25^{o} than at 50^{o} (Dodds et al. 1956). Thus, increase in isotope fractionation with increase in temperature cannot be a function of the availability of carbon. We believe the effect is probably due to the involvement of different temperature-contolled rate-limiting processes, either in activation or transfer of carbon dioxide by enzyme-bound moieties. In nature, different algal species could have optimum enzyme-substrate reactions at a variety of temperatures, pH and substrate levels, hence yielding different isotope effects even under identical conditions of growth.

For comparison, samples of hot spring algae were measured from environments of known pH and temperature. A mat of C. calderium (collected by J. Oehler, June 1971) from Nymph Creek in Yellowstone Park actively growing in water at 44^{o}C and pH 2.7, was analysed some weeks after collection and yielded $\delta C^{13} = -12.3‰$ or $\Delta\delta C^{13} = -5.3‰$ (assuming air $CO_2 = -7‰$). This value is less than that measured for air-grown cells in the laboratory.

Algal mats from the Orakei Korako geothermal area of New Zealand (see Kaplan, 1956) were collected by E. Lloyd of D.S.I.R. around springs, ponds and geysers during 1960 and 1961 at different seasons. The water temperature at the source and that in contact with the algal mats was measured. The pH was measured after permitting the water to cool to atmospheric temperature. The algal mats were then air-dried by spreading them out in a warm room. All samples were first treated with 1N HCl to dissolve carbonate, before combustion for isotope measurement.

It is apparent that at the high pH in this environment, bicarbonate will predominate. The spread in δC^{13} is from -11.1‰ to -23.8‰ over a temperature of growth range of 30° to 63°C. However, a glance at Table 2 indicates that there is no direct relationship between temperature of growth and the δC^{13} of the algae. Paradoxically, the greatest enrichment in C^{13} occurred at the highest temperature of growth. Unfortunately, the δC^{13} of the dissolved HCO_3^- is not known, but one may assume that it is reasonably constant for all the pools, as the environment of growth is localized and source of water is probably the Waikato River.

These preliminary data suggest that caution must be exercised in interpreting C^{13}/C^{12} data on naturally-occurring organisms and their degraded products (in soil or sediment), if they are to be used in interpreting environmental conditions, especially temperature of growth.

However, one may speculate from the results, that unusual enrichments in C^{12} occasionally found in kerogen from Precambrian rocks (Hoering 1967; Smith et al. 1970; Oehler et al. 1972),

may have originated in hot spring algae growing in acid waters under a high tension of CO_2.

ACKNOWLEDGEMENT

This work was supported by grants from NASA (#237-62; NGR 05-007-221) to UCLA and by a special grant from the Hebrew University to J. Sechbach. We wish to thank Professor W.F. Libby for his interest in this study and to Ed Ruth and Chari Petrowski for making the mass spectroscopy measurements.

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Smith, J.W., J.W. Schopf and I.R. Kaplan. 1970 Extractable organic matter in Precambrian cherts. Geochim. Cosmochim. Acta, 34: 659-675.

Table 1. Carbon isotope fractionation, $\Delta\ \delta C^{13}$,* by laboratory grown C. cyanidium in the presence of air or CO_2 at various temperatures

	Temperature °C of growth							
	25 — 26		40	45			50	
	Age in days of culture							
	14	41	11	14	17	18	14	26
Substrate:								
CO_2	-5.1[1‡]	-19.0[3]	-17.0[4]	-16.5[1]	--	-24.0[3]	-19.3[1]	-16.6[4]
	-11.0[3]							
Air	-12.3[1]	-11.0[3]	-14.9[4]	---	-13.0[2]	-11.0[3]	-13.8[1]	-14.8[4]
	-12.0[2]							

*$\Delta\ \delta C^{13} = \delta C^{13}$ sample - δC^{13} substrate

‡ Experiment number

Table 2. C^{13}/C^{12} ratios of *Cyanidium* lipid extracted from cells grown under pure CO_2 and air (0.03% CO_2) at different temperatures

Temperature (^{o}C)		25		45	
Age (days)		41		18	
		δC^{13} (‰)			
Fraction		Lipid	Residue	Lipid	Residue
Condition	CO_2	-18.9	-17.0	-24.5	-23.3
	Air	-14.1	-11.2	-19.6	-10.7

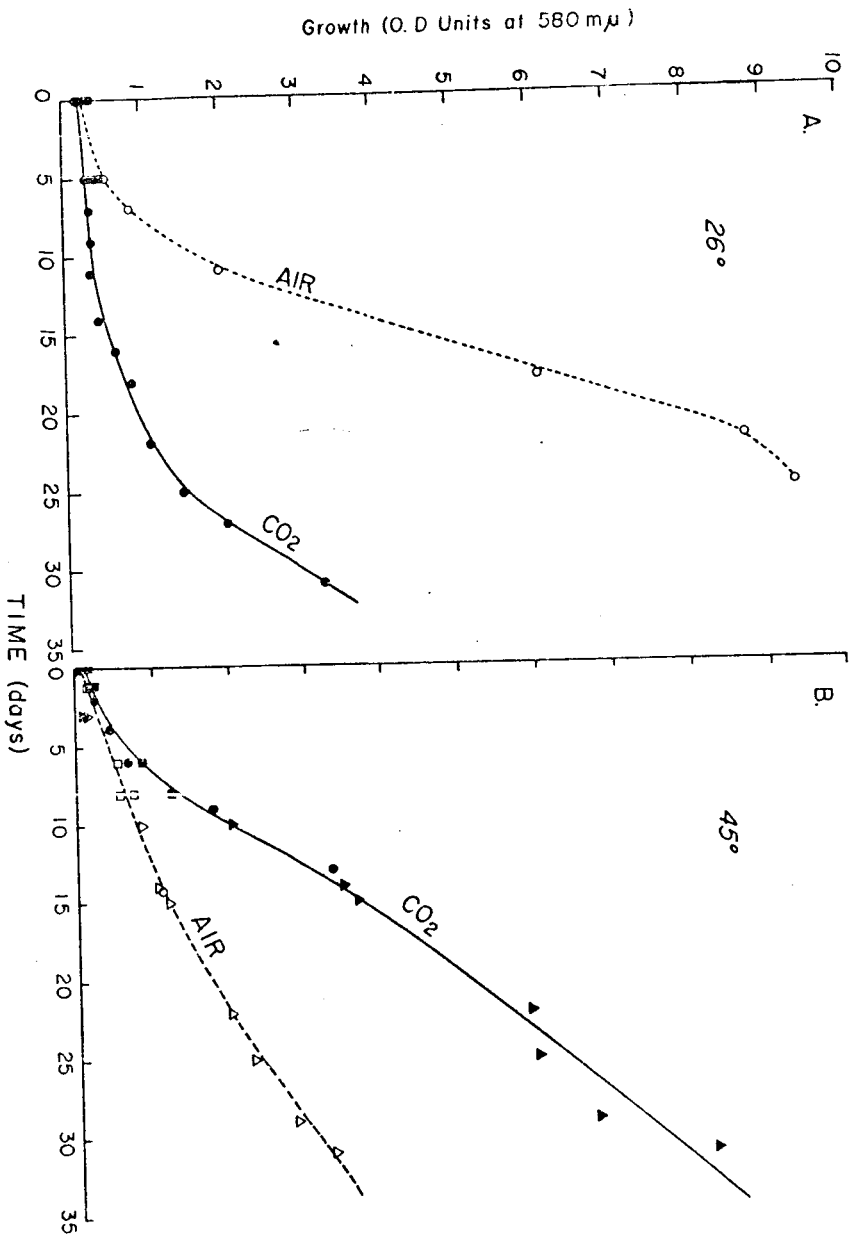
Table 3. Algal mats from Orokei Korako (New Zealand) Hot Springs

Sample #	Date Collected	Site	Spring T (°C)	T sampling site	pH	δC^{13} (‰)
1	3-21-60	I	99.8	44	9.75	-19.61
3	3-21-60	I	99.8	44	9.75	-18.26
34	5-25-60	I	99	30.5	9.6	-20.00
74	8- 2-60	I	104	35	9.56	-20.81
139	1-18-61	I	--	--	--	-19.89
7	3-21-60	III	98	63	9.3	-22.81
38	5-25-60	III	97.5	--	9.32	-14.6
76	8- 2-60	III	98	60.5	9.22	-19.47
146	10-11-60	III	--	63.7	--	-19.89
13	3-21-60	VI	75.5	63.7	8.46	-23.80
14	3-21-60	VI	---	43	--	-11.40
42	5-25-60	VI	73	56.5	8.38	-18.38
79	8- 2-60	VI	74	--	--	-15.97
105	10-11-60	VI	75	--	8.5	-15.41
149	1-18-61	VI	--	40-30	--	-11.09

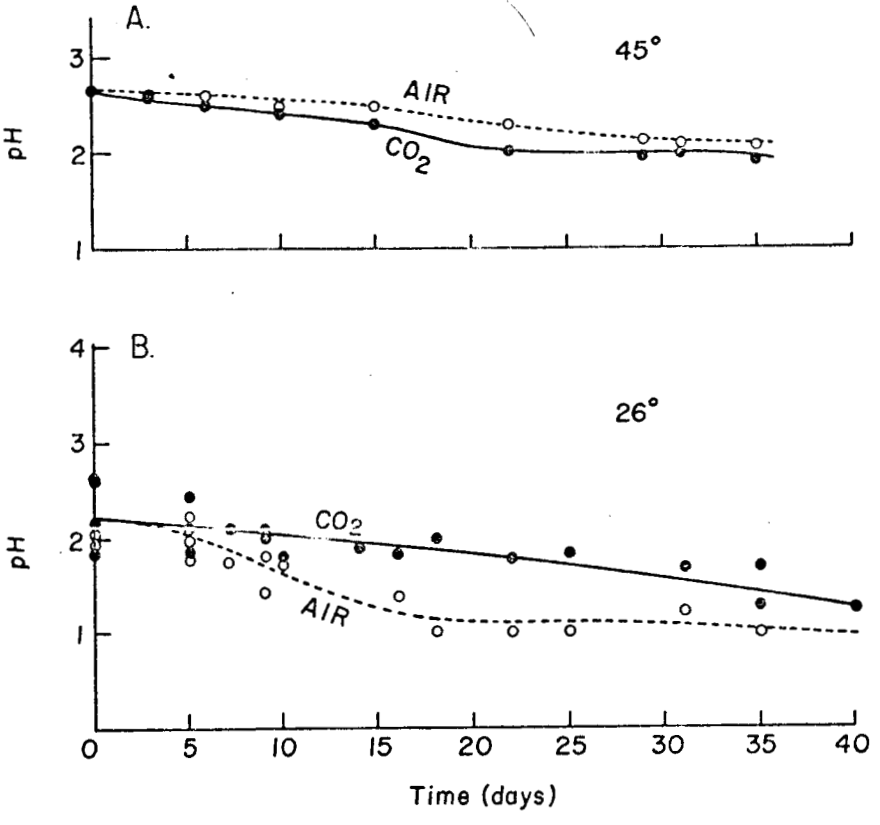
LEGENDS FOR FIGURES

Figure 1 Growth of *Cyanidium caldarium* as a function of CO_2 (solid figures) or air (open figures) at room temperature (A) and at 45° (B). Growth values are expressed in units of O.D. at 580 mμ (which gives a relatively accurate picture of the cell mass at this wavelength.

Figure 2 The pH modification of the nutrient media during growth of *Cyanidium caldarium* cells under CO_2 (_____) or air (---------) at 45° (A) and 26° (B).


Growth (O. D Units at 580 mμ)
A.
26°
AIR
CO2
B.
45°
CO2
AIR
TIME (days)

The pH modification of the nutrient media during the growth of *Cyanidium* cells under CO_2 (——) or air (----) at 26° and 45°.



PERYLENE AND ITS GEOCHEMICAL SIGNIFICANCE*

by

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ABSTRACT

Perylene was found in a variety of marine sediments, a shale and in peat. It is suggested that its precursors arise predominantly from land organisms and are carried into oceanic traps along with detrital minerals. When rates of deposition are fast, and reducing conditions are established within the sediment, biogenic pigment precursors of perylene are converted to the polycyclic aromatic hydrocarbon, which is then stabilized by π-bonding with metals and protected from degradation.

INTRODUCTION

Perylene (see Fig. 1) has been found in the aromatic hydrocarbon fractions of some sediments and certain petroleum high boiling fractions (Schnurmann et al. 1953; Carruthers and Cook 1954). More recently, perylene has been reported in soil (Blumer 1961), peat (Gilliland and Howard 1960; Bergmann, Ikan and Kashman 1964), marine sediments (Orr and Grady 1967), and fresh water lake sediments (Hodgson et al. 1968a). In most cases, only semi-quantitative data have been reported.

This study was prompted by the fact that the results and conclusions, reported by Orr and Grady (1967) may be questioned, on the basis of possible oil seeps in nearby Santa Barbara Channel. As polycyclic and other aromatic compounds are generally not detectable in recent marine sediments, and because perylene can be measured in low concentrations (< 1.0 ppm), it was decided to re-investigate the occurrence of perylene in a variety of marine sediments.

EXPERIMENTAL

Sample description

All the marine samples studied consist of clay minerals as a major component. The interstitial waters were studied by our group extensively except for the Bandaras Bay (B.B.-1) sample. For the present study, it is important to establish the redox state of the sediment at the time of deposition and at present. In the case of the Santa Barbara samples, Core 3

of the Saanich Inlet, and the San Pedro Martir Basin, the negative Eh and sulfate reduction are evident from the surface down the cores, while our samples from Tanner Basin (T_1 and T_2) indicate no sulfate reduction in the first meter, but an extensive reduction below that depth. Core J-26 (JOIDES) exhibits another inconsistency, while the Eh recorded is positive, there is sulfate reduction from 100 m. to 230 m. Therefore, we suggest using the total organic matter and the preservation of some specific organic tracers as a criterion for determining the redox history of a given sample (see Results and Discussion Section). Using this criterion, we labelled each sample in Table 1 according to its apparent redox state, either "R" (reducing) or "O" (oxidizing).

More detailed descriptions of the samples and their interstitial waters are given in the following references: Santa Barbara, Tanner and San Clemente Basins (Emery 1960), Saanich Inlet (Brown et al. 1972), Gulf of Mexico and Vema Fracture Zone (JOIDES, holes 3 and 26; Kaplan et al. 1972); Bandaras Bay (Drever 1971).

Perylene: Qualitative and Quantitative Determinations

The extraction and silicic acid chromatography procedures employed for this study, were described previously (Brown et al. 1972, Kaplan et al. 1972).

Fraction I, separated on silicic acid column with hexane as eluant (see path A in flow chart; Fig. 2), contains most of the perylene. In some cases, a portion trails into the benzene fraction (II), and before re-chromatographing on silica

gel both fractions should be checked as to their UV-Vis spectra. In most samples when carotenoids are present, the second chromatography on silica gel is necessary (see Fig. 3) and will be carried on the combined fractions I and II. Elution with hexane will separate the carotenoids with the saturated hydrocarbons and hexane:benzene (1:1) will elute the perylene (which displays an intensive fluorescence) with some other aromatics. For best separation, and to avoid a mixture of compounds that absorb in the same region of the UV-Vis as perylene, the change from hexane elution to benzene should be gradual (5,10,25,40,50%). Although this method gives good qualitative results, in some cases it fails to give quantitative values because other aromatics cover the same region of UV-Vis absorption (sample J-3-34 is a good example). In the UV technique for identification and quantitative determination, one should note that of the four typical peaks in perylene's UV-spectrum, the highest $\lambda^{\text{Benzene}}_{\text{Max.}}$ 440 mμ was selected, $\varepsilon = 4.06 \times 10^4$ l/mole/cm (Schnurmann et al. 1953).

For those samples where the UV method could not be effectively employed, we applied GLC and GLC-mass spectrometry. This technique proved to be simple and precise. In this method, after extraction of the sample, only one liquid column chlromatographic separation is required. The extract is evaporated to near-dryness and placed on Florisil column (see path B flow chart, Fig.2). The first fraction eluted with hexane contains aliphatic hydrocarbons and the second, eluted with benzene, contains the aromatics. The benzene fraction is resolved by gas-chromatography on a 6'X1/8" Ap-L (or OV-17) column, temperature programmed from 200° to 300°C at a

rate of 12°/min. (see Fig. 4). For further identification, a standard perylene sample is coinjected.

Samples with the highest perylene concentrations were checked by a combination of GLC and medium resolution mass-spectrometry (CEC 21-491). Whereas, the GLC detection limit is 0.01 μg/100 gr. of dry sediment, the combined technique could not be applied for samples containing less than 25 μg/100 gr (if the amount of sediment extracted is <100 gr.), because of column background and mass-spectrum sensitivity limits. By comparison with standard perylene, the mass spectrum shows that the extracted perylene is at least 95% non-substituted and the 5% probably alkyl derivatives (see Fig.5).

All packing materials for liquid chromatography were washed in the reversed solvent polarity order of use (methanol, benzene, hexane) and dried at 130-150°C for four hours. The packing materials used were: (1) Silicic acid (for lipid chromatography) Bio-SIL-HA, -324 mesh (Bio. Rad. Laboratories); Silica gel, 30/60 mesh (Applied Science Laboratory Inc.); Florisil, FX-285-1, -100 mesh (Matheson Coleman & Bell).

RESULTS AND DISCUSSION

The results of the present investigation are summarized in Table 1, and compared with those of Orr and Grady (1967). In the course of this study, a variety of lithified and unlithified sediments were studied, representing different geographic distribution, terrestrial, neritic, and bathyl environments and depth of burial. The ages of these sediments range from very recent to hundreds of millions of years.

It is apparent that age is not an important criterion for the presence or absence of perylene. From the analysis of 12 deep sea sediment samples (JOIDES) perylene was only detected in samples from J-26 (JOIDES Hole 26) and in one sample from the Gulf of Mexico (J-3-34). It was present in near-shore sediments such as the Saanich Inlet fjord, British Columbia and Bandaras Bay, which is fed by flood waters from the Rio Ameca. We were also able to confirm the results of Orr and Grady (1967) that perylene is absent in the sediment of Tanner Basin (approximately 150 Km off the southern California shore). Relatively high concentration of perylene was extracted from a recent English (Glastonbury) peat.

Orr and Grady (1967) explained the presence of perylene in the Santa Barbara sediments as a result of preservation of organic constituents rapidly deposited under anaerobic conditions from shallow oxygen-poor water. They assumed that the precursors for the hydrocarbon, possibly hydroquinones (shown in Fig. 1), arise from pigments in marine organisms. This would involve step-wise hydrolysis and reduction of the C=O bond leading to dehydration of the phenols (C-OH) to give the aromatic structure as originally proposed by Blumer (1965). Unfortunately, no precursors of perylene or other polycyclic aromatics extracted from recent sediments, (1.12-benzperylene and coronene; Meinschein 1959) have been recognized. It is known, however, that insects (Cameron et al. 1964) and fungi (Thompson 1957; Allport and Bulock 1960) do contain several pigments which could be perylene precursors. Hodgson et al. (1968b) claim that the UV spectrum of some pigments from swiss chard, spinach and lettuce closely resembles

that of perylene found in petroleum and sediments. Furthermore, Bergmann et al. (1964) report the isolation of crystalline perylene from peat, derived from land plants and their associated biota.

It appears that the necessary conditions for the formation and preservation of perylene in marine sediments are: (a) source of material (b) deposition history (rapid or slow: mode of transport in water column or turbidity currents, etc.) (c) preservation history (redox conditions, temperature, complexing, etc.). Orr and Grady (1967) believe that the last two are the controlling factors based on a marine origin of the biological precursors. An alternative explanation can be offered, that the precursors of perylene originate on land and are transported into the ocean. They will only survive if transportation is sufficiently rapid to prevent degradation of biochemicals. For example, 4,9-dihydroxyperylene-3,10-quinone (II) (see Fig. 1) which is considered a possible precursor of perylene, is much more vulnerable to oxidation than perylene, itself. Transformation of these precursors (see Fig. 1) into the aromatic polycyclic hydrocarbons will occur if deposition takes place in a reducing environment. Interpretations based on the environment of deposition and preservation are the same as those suggested by Orr and Grady, but we believe that the source of the precursors may be largely or entirely terrigenous.

Evidence for the presence of land-derived organic components is seen in two properties of the organic matter. First, in all samples where perylene was detected, long-chain hydrocarbons

ranging from C_{27} to C_{31} were abundant (see Fig. 4 and 6). Furthermore, the carbon preference index (CPI) of these hydrocarbons was >1.3. In the case of hydrocarbons from Glastonbury peat, the CPI is above 4, (see Fig. 6A). However, in the case of the Tanner Basin (T_1 and T_2) and other reducing sediments such as the San Pedro Martir Basin (GC-15-25), hydrocarbons in the range C_{18} to C_{25} are most abundant, and there is no apparent odd-over-even predominance (see Fig. 6B). Second, C^{13}/C^{12} measurements of the organic carbon compounds in samples containing perylene indicates some component of higher plant material is present. Marine-derived organic matter generally has δC^{13} values of -19 to -20 ‰, whereas land-derived organic matter usually yields δC^{13} values between -25 and -28 ‰. The combination of the above two criteria help identify relative sources of organic matter. It is significant to note that samples analysed by Meinschein (1959) in which polycyclic aromatic hydrocarbons were detected, all came from near shore (Gulf of Mexico) and, apparently, so did those in which Hodgson (1968a) reported perylene-rich ("type I") aromatics.

The argument of Orr and Grady (1967) for absence of perylene in Tanner Basin sediment because of the depth of the water column is reasonable; however, it does not explain the presence of perylene in sample J-3-34 from hole 3 (JOIDES) or in the samples from Hole 26. In both these cases, it is apparent that extensive turbidity currents have been responsible for deposition. In the case of Hole 26 (Vema Fracture Zone) the source of the sediment was probably the Amazon River, 1200 Km away.

In support of the above argument is the fact that other highly

unstable molecules, such as carotenoids and chlorophyll-derived chlorins, were present in Tanner Basin and the Gulf of California sediments. If oxidation, either in the water column during deposition, or at the sediment-water interface was extensive, these molecules would have been degraded. Furthermore, the study of the San Pedro Martir Basin sediments indicates a very close resemblance to the marine-type organic matter in the Tanner Basin. Sample GC 15-25 is very rich in pheophytine and carotenoids, but no perylene was found in this core despite the fact that sulfate is reduced rapidly at the surface. Once perylene forms, it can be stabilized by π-complexing with transition metals (Clar 1964). Hence, even when carotenes can no longer be detected in older sediments, due to degradation (or complexing into humic acids or kerogen-like compounds) perylene will persist once it is formed.

Analysis of two organic-rich Miocene shales from Southern California (Monterey shale and Nicholas Formation shale) indicates the absence of carotenoids in both. However, the Monterey shale contains perylene (150 μg/100 gr) and a relatively high concentration of chlorins ($\sim$100 μg/100 gr) whereas Nicholas shale contains no perylene or chlorins, but small amounts of a porphyrin ($7X10^{-3}$ μg/100 gr., probably Fe-porphyrin) indicating oxidation of organic matter. Both these shales contain long-chain aliphatic hydrocarbons with CPI values higher than 2.0.

It therefore appears that rapid introduction of sediment, probably by turbidity currents will transform relatively high contents of detrital material into neritic as well as bathyal traps. This rapid deposition will allow reducing conditions to be established and convert pigment precursors, possibly by

hydrolysis and reduction to relatively stable perylene. Where oxidizing conditions exist at the surface of the sediment, or where the source of organic matter is entirely (or predominantly) marine, perylene will not form in the sediment column during diagenesis.

As stated earlier, perylene is present in small amounts in some petroleum deposits. Accroding to the principles expounded by Yen et al. (1961), the molecular configuration of perylene is not compatible with the polynuclear network composed of naphthalene rings and other peri-polycyclic aromatics, therefore it could not be synthesized under the same conditions. Thus, perylene never becomes an important constituent in aromatic dominated petroleum. Its presence in petroleum arises from extraction out of the source shale or sediment through which oil migrates.

ACKNOWLEDGEMENTS

I thank M.J. Baedecker for the hydrocarbon fractions from the Saanich Inlet, C. Petrowski for the carbon isotope measurements and I.R. Kaplan for helpful discussions and review of the manuscript. Coring in the Tanner Basin was funded by R. Hessler (Scripps vessel R/V Alexander Agassig) and collected by E.K. Kalil and R. Sweeney. The Bandaras Bay mud was presented to us by J.I. Drever and the San Pedro Martir Basin core was collected by J. Bischoff (USC vessel R/V Velero, core 833221).

This research was supported by NASA grant NGR 05-007-221.

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TABLE I

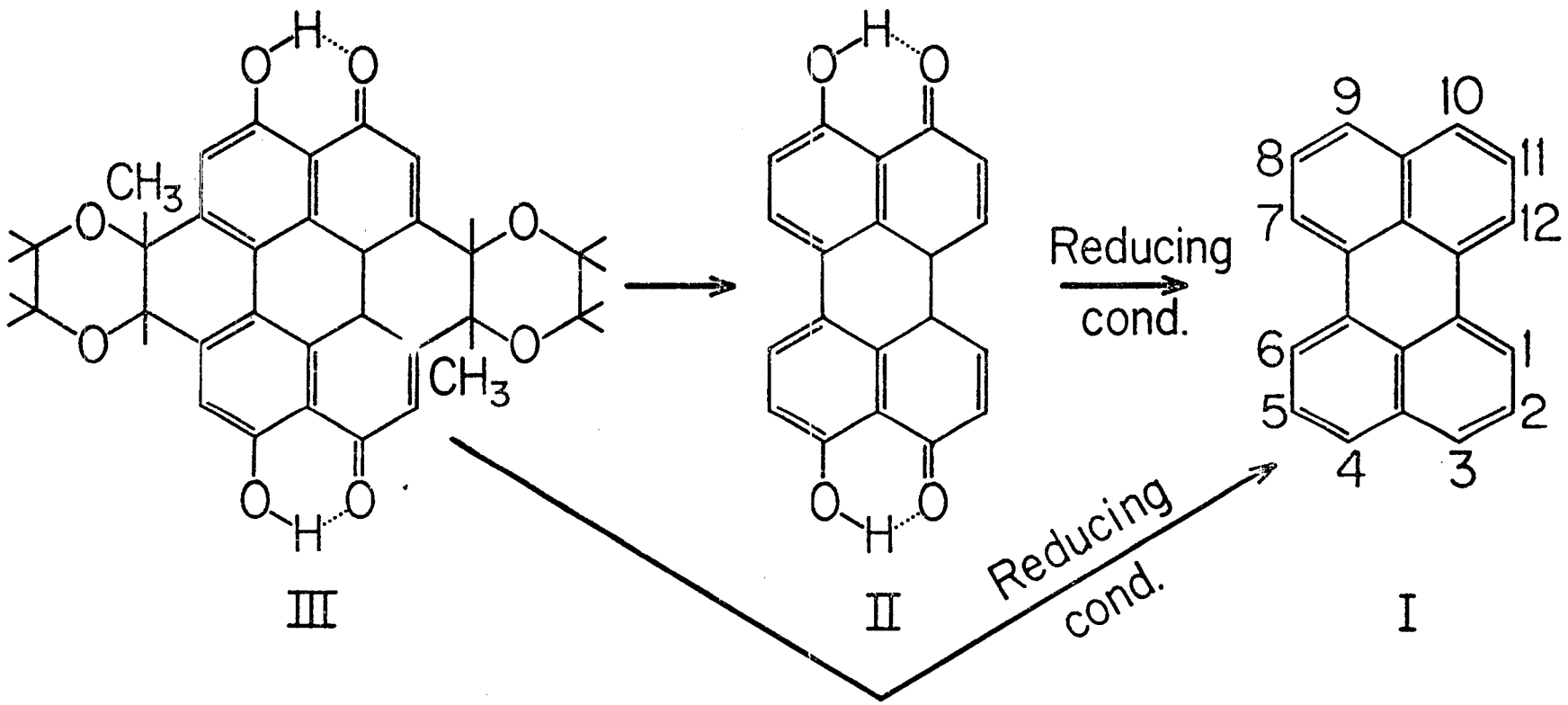
Sample Code Number	Location & Place	Depth in (m)* below sea floor	Sea Floor depth (m)	Oxidation state of sediment	% Org. C.	$\delta C^{13}/C^{12}$ (‰)	PERYLENE+			
							μg/100 g of dry sediment		% of org. C X10^2	
							GLC	UV-Vis	GLC	UV-Vis
	SANTA BARBARA BASIN									
AHF 2622	34° 13.5'N 120° 01.9'W	0 -0.5	600	R	5.8	-21-23	--	7.8	--	0.013
AHF 3503	34° 13.5'N 120° 02.2'W	0.5-0.8	600	R	5.8	-21-23	--	8.6	--	0.015
FRL 163G	34° 11.0'N 120° 03.0'W	0.8-1.5	580	R	5.8	-21-23	--	24.4	--	0.042
AHF 2622	34° 13.5'N 120° 01.9'W	1.2-1.4	600	R	5.8	-21-23	--	26.0	--	0.045
AHF 3504	34° 08.9'N 120° 01.6'W	0 -0.5	493	O (?)	5.8	-21-23	--	<0.02	--	--
	TANNER BASIN									
AHF 4696	32° 57.4'N 119° 44.5'W	0- 0.4	1510	R	11.7	-20.5	--	<0.1	--	--
AHF 4696	32° 57.4'N 119° 44.5'W	0.6- 1.5	1510	R	11.7	-20.5	--	<0.1	--	--
AHF 4696	32° 57.4'N 119° 44.5'W	3- 3.3	1510	R	11.7	-20.5	--	<0.1	--	--
T-1	32° 56.2'N 119° 43.5'W	0- 0.2	1472	R	11.1	-23.0	<0.01	<0.1	--	--
T-2	32° 56.2'N 119° 43.5'W	0.9- 1	1472	R	11.2	-22.6	<0.01	<0.1	--	--
	SAN CLEMENTE BASIN									
AHF 3669	32° 37.7'N 118° 07.5'W	Surface	2060	R	5.7	-21.5	--	<0.02	--	--
	SAN PEDRO MARTIR BASIN									
Gulf of California										
GC 15-25	28° 20'N 112° 23'W	0.15-0.25	883	R	4.75	-21.0	<0.01	<0.1	--	--
Core 3	**SAANICH INLET (B.C.)**									
3/0-15	48° 30.40'N 123° 30.06'W	0- 0.1	200	R	3.87	-20.2	34.32	32.9	0.088	0.085
3/190-200	48° 30.40'N 123° 30.06'W	2- 2.1	200	R	--	-20.3	74.10	73.0	--	--
3B/1710-1740	48° 30.40'N 123° 30.06'W	17.1-17.4	200	R	2.82	-21.6	167.58	165.9	0.590	0.588
3B/3450-3480	48° 30.40'N 123° 30.06'W	34.5-34.8	200	R	2.53	-22.5	237.00	231.0	0.940	0.913
Hole 3 "JOIDES"	**GULF OF MEXICO**									
J-3-34	23° 01.8'N 92° 02.6'W	34	3747	R ⇋ O	1.11	-26.6	1.20	n.c.q.	0.01	--
J-3-209	23° 01.8'N 92° 02.6'W	209	3747	O	0.82	-21.7	<0.01	--	--	--
J-3-324	23° 01.8'N 92° 02.6'W	324	3747	O	0.47	-22.1	<0.01	--	--	--
J-3-534	23° 01.8'N 92° 02.6'W	534	3747	O	0.47	-22.2	<0.01	--	--	--

Hole 26 "JOIDES" VEMA FRACTURE ZONE											
J-26-100	10°53.55'N	44°02.57'W	100	5168	R	0.87	-25.3	18.46	15.9	0.212	0.182
J-26-230	10°53.55'N	44°02.57'W	230	5168	R	1.00	-27.0	33.13	33.5	0.331	0.335
J-26-478	10°53.55'N	44°02.57'W	478	5168	O	0.51	-25.2	16.07	14.9	0.316	0.292
	BANDARAS BAY										
Surface mud											
B.B-1	20°39.9'N	105°16.1'W	Surface	94	R	1.64	-23.41	25.4	25.4	0.155	0.155
England (Avalon) GLASTONBURY PEAT											
P-EG	51°09'N	2°45' W	--	--	R	53.3	-28.1	301.00	315.0[a]	0.056	0.059

a = very rich in other aromatics
n.d. = none detected
n.c.q. = none calculated quantitatively
\+ = Perylene detection limit for GLC 0.01 μg/100 gr; for UV-Vis 0.1 μg/100 gr.
R = reducing
O = oxidizing
* = depth reported for the JOIDES cores will be ±0.5 m.

All AHF and FRL coded cores data from Orr and Grady (1967).

LIST OF FIGURES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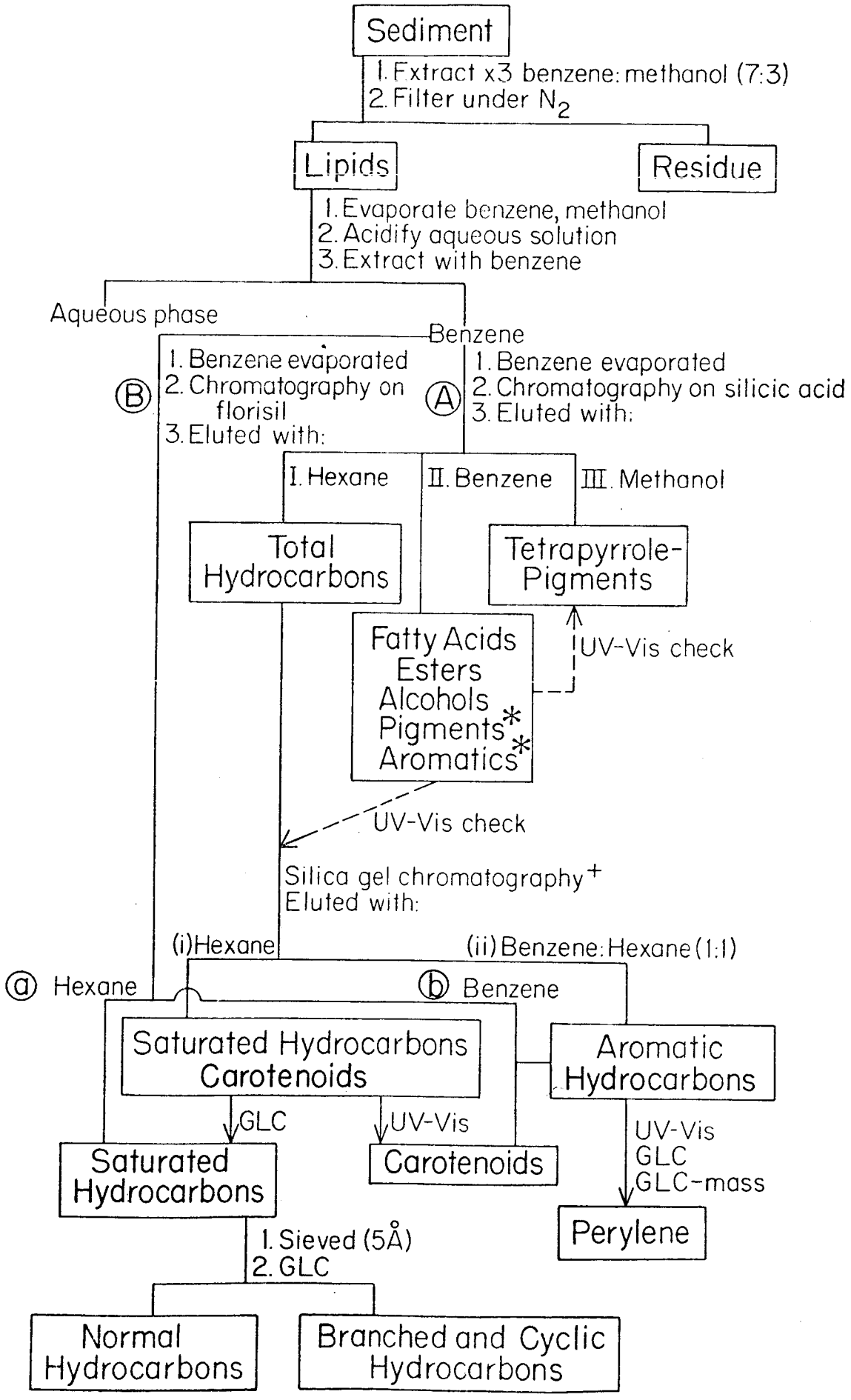
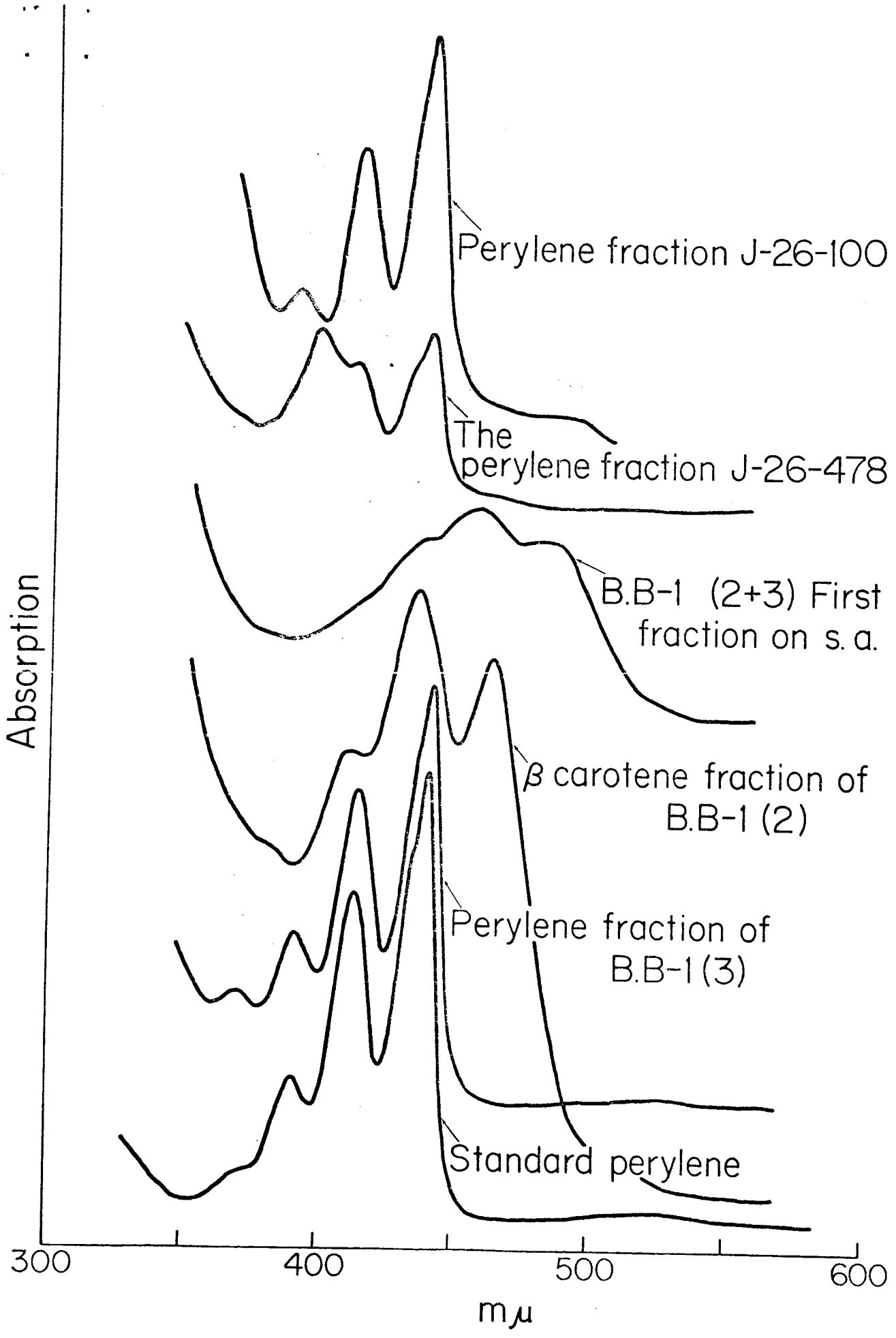
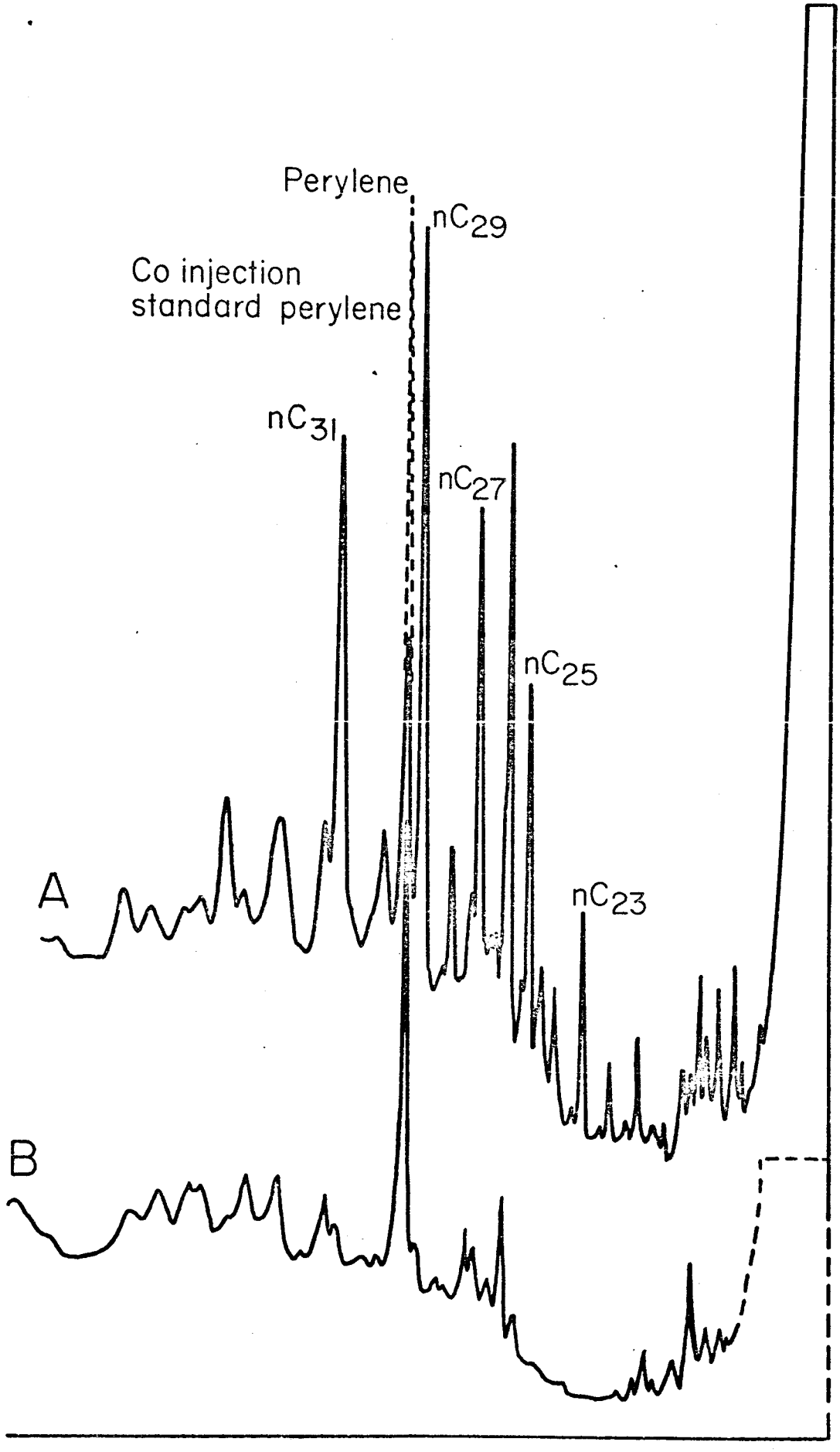
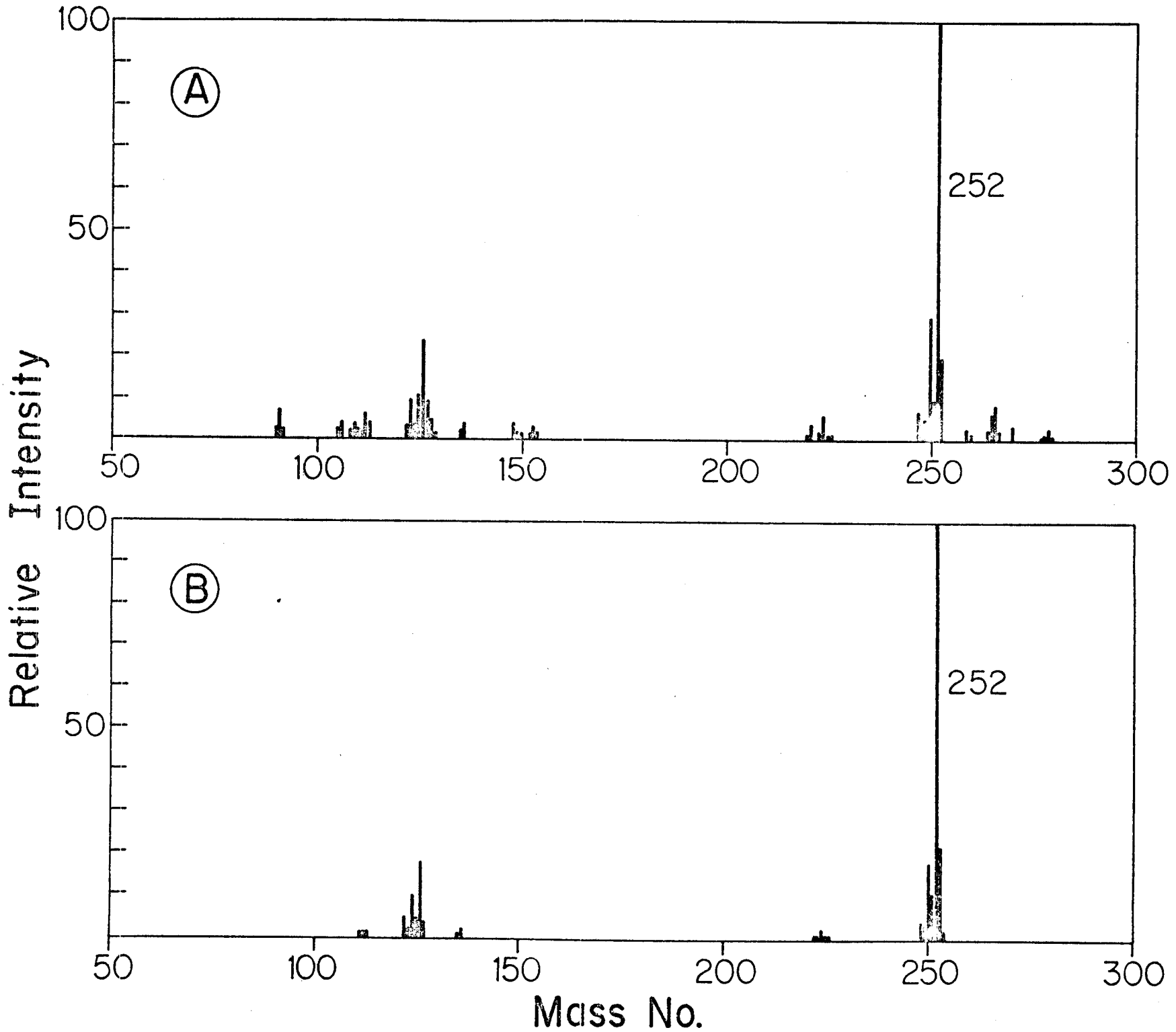
CH3
Reducing
cond.
Reducing
cond.
9
10
8
11
7
12
6
1
5
2
4
3
III
II
I


Sediment
1. Extract x3 benzene: methanol (7:3)
2. Filter under N_2
Lipids
Residue
1. Evaporate benzene, methanol
2. Acidify aqueous solution
3. Extract with benzene
Aqueous phase
Benzene
B
1. Benzene evaporated
2. Chromatography on florisil
3. Eluted with:
A
1. Benzene evaporated
2. Chromatography on silicic acid
3. Eluted with:
I. Hexane
II. Benzene
III. Methanol
Total Hydrocarbons
Tetrapyrrole-Pigments
Fatty Acids
Esters
Alcohols
Pigments*
Aromatics*
UV-Vis check
UV-Vis check
Silica gel chromatography+
Eluted with:
(i) Hexane
(ii) Benzene: Hexane (1:1)
ⓐ Hexane
ⓑ Benzene
Saturated Hydrocarbons
Carotenoids
Aromatic Hydrocarbons
GLC
UV-Vis
Saturated Hydrocarbons
Carotenoids
UV-Vis
GLC
GLC-mass
Perylene
1. Sieved (5Å)
2. GLC
Normal Hydrocarbons
Branched and Cyclic Hydrocarbons


Perylene fraction J-26-100
The perylene fraction J-26-478
B.B-1 (2+3) First fraction on s. a.
β carotene fraction of B.B-1 (2)
Perylene fraction of B.B-1 (3)
Standard perylene
Absorption
300
400
500
600
mμ


Perylene
nC29
Co injection
standard perylene
nC31
nC27
nC25
nC23
A
B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50
A
252
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300
Relative Intensity
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50
B
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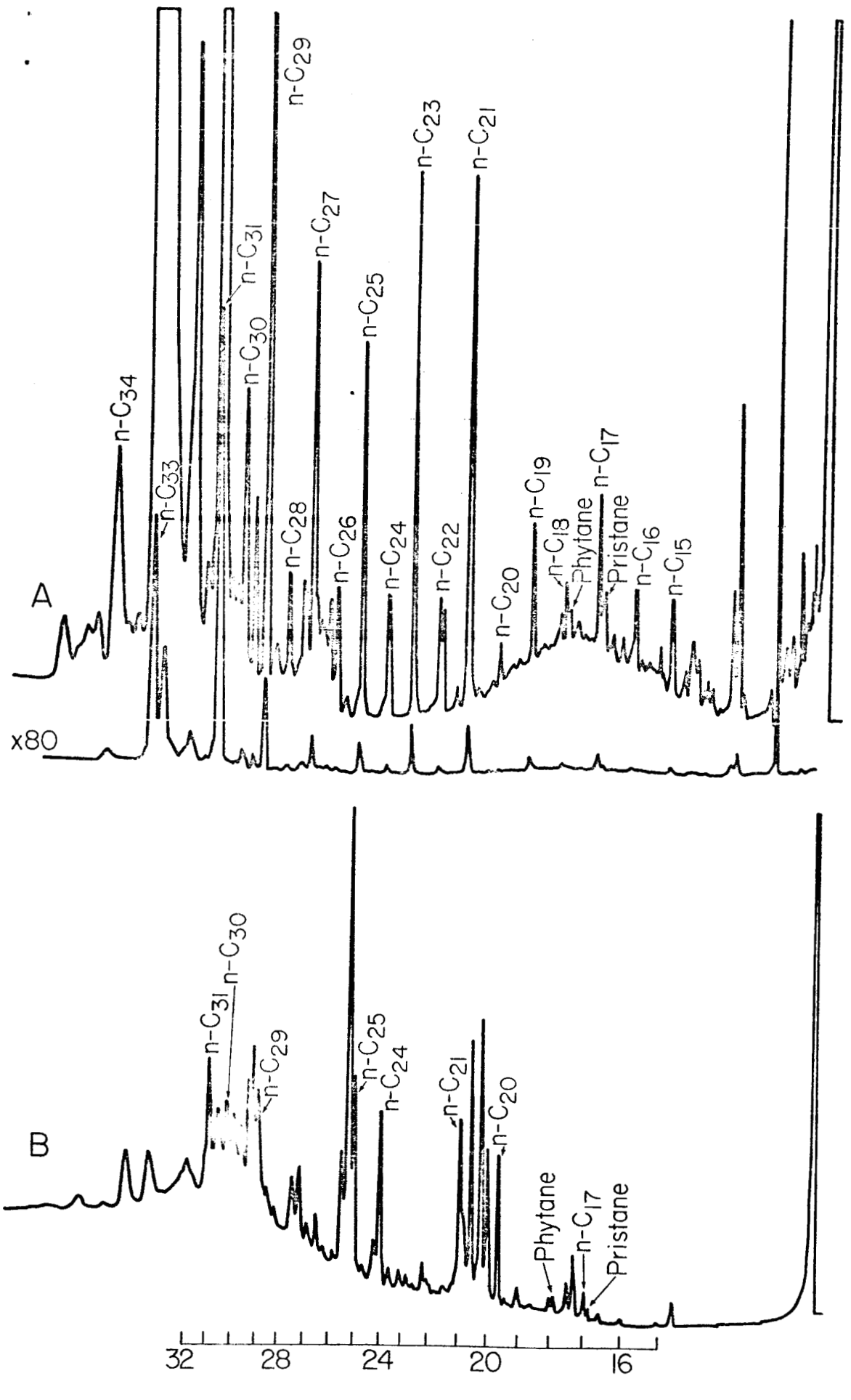
50
100
150
200
250
300
Mass No.


A
x80
n-C34
n-C33
n-C31
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n-C18
Phytane
n-C17
Pristane
n-C16
n-C15
B
n-C31
n-C30
n-C29
n-C25
n-C24
n-C21
n-C20
Phytane
n-C17
Pristane
32
28
24
20
16
Carbon number

THERMAL DEGRADATION OF BLUE-GREEN ALGAE AND BLUE-GREEN ALGAL CHLOROPHYLL

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Abstract--The thermal degradation of blue-green algae (Lyngbya) and blue-green algal chlorophyll have been studied at temperatures ranging from 25 to 300°C for periods ranging from ½ hour to 2000 hours under both oxygenic and anoxic conditions. An irreversible series of time- and temperature-dependent color changes occurred in both the intracellular pigments and the extracellular sheaths of the heated algae. These sequential color changes are comparable to those resulting from thermal alteration of plant microfossils, especially palynomorphs, which have been used for mapping eometamorphic facies. Extrapolations based on the measured time-temperature relationships of these color changes are consistent with pigment changes observed in Holocene and Tertiary organic matter, suggesting that these experimental results may have geochemical applicability. Observed degradation pathways of intracellular chlorophyll differ from those reported for extracellular chlorophyll and its derivatives (ORR et al., 1958); intracellular pigments apparently become grafted onto cellular polymers, a process that presumably occurs also during natural diagenesis. These results suggest that formation of pigment-polymer complexes may be an important diagenetic process affecting the types and distribution of pigments in sediments and sedimentary rocks.

INTRODUCTION

It has long been recognized by paleobotanists and coal petrographers that both the fidelity of cellular preservation and the color of carbonaceous plant fossils and particulate organic matter (kerogen) preserved in ancient sediments vary as a function of metamorphic history and degree of coalification. In recent years, palynologists and petroleum geologists have become increasingly interested in the continuum of color changes (generally from yellow, through various shades of red and brown, to black) accompanying the metamorphic alteration of kerogenous organic matter, especially that comprising organically preserved plant microfossils. Of the various factors apparently contributing to such alteration, temperature has been widely regarded as dominant. Thus, temperatures extrapolated from known geothermal gradients or measured in boreholes or in short-term laboratory heating experiments have been correlated with palynomorph color to yield "thermal alteration indices"; these color scales have been used for mapping eometamorphic facies and for predicting the potential oil and gas productivity of fossiliferous strata (GUTJAHR, 1966; STAPLIN, 1969; BURGESS, 1970; CORREIA, 1971; WILSON, 1971).

Although temperature is no doubt important in producing palynomorph color changes, organic geochemical studies have suggested that time should also be considered a major factor

defining the thermal stability of organic materials (e.g., ABELSON, 1959, 1967; EGLINTON, 1969); given sufficient time, geochemical degradation of organic compounds can apparently occur at quite low temperatures. However, the possible role of time or of time-temperature relationships in the alteration of kerogenous microfossils has been largely neglected. Moreover, those studies that have considered time-temperature as an integrated component have been concerned chiefly with the stability of simple organic compounds in the form of pure solids or in dilute, aqueous solution (e.g., ABELSON, 1959). Thus, virtually no experimental data are available to suggest the geochemical fate of organic compounds that enter the geologic record as components of intact microorganisms (and are thus subjected to an organic-rich, highly reactive, intracellular environment) or to indicate the relative importance of time and temperature in the organic preservation of such incipient microfossils.

Our interest in these problems stems from a continuing study of the mode and effect of preservation of microorganisms by silica permineralization under controlled, laboratory conditions (OEHLER and SCHOPF, 1971). These investigations have shown that microscopic blue-green algae can be "articifically fossilized" as structurally intact organic residues by a series of stages that seem analogous to those producing bedded, fossiliferous cherts in nature; varying

degrees of fidelity of preservation, corresponding in color and morphology to varying stages of "carbonization" observed in naturally occurring algal microfossils, have been produced. To provide data with which to interpret the chemical changes that occur during "artificial fossilization," and to serve ultimately as a partial basis for comparison of the chemistry of living and fossil cyanophytes, we have investigated experimentally the thermal alteration of intact blue-green algae at temperatures ranging from 25 to 300°C and for periods ranging from $\frac{1}{2}$ hour to 2000 hours under both oxygenic and anoxic conditions. Related studies have been made of various components of these microorganisms (e.g., empty sheaths, apochlorotic filaments, and extracted chlorophyll _a_). These investigations provide new data regarding the kinetics of processes resulting in color changes during thermal alteration of blue-green algae, the chemistry of some processes involved in such changes, and the pathways of degradation of intracellular, as compared with extracellular, chlorophyll.

EXPERIMENTAL PROCEDURE

The relatively broad, sheath-enclosed, blue-green alga _Lyngbya_ (Oscillatoriaceae) was selected for these experiments because (_i_) its large size and filamentous habit facilitate handling; (_ii_) it is the subject of continuing research on artificial fossilization (OEHLER and SCHOPF, 1971); and (_iii_)

Lyngbya-like cyanophytes have an extensive fossil record extending into the late and middle Precambrian (BARGHOORN and TYLER, 1965; SCHOPF, 1968; SCHOPF and BLACIC, 1971).

Short-term heating experiments

1) Twenty 0.1 gm samples of Lyngbya filaments were heated for thirty minutes each at temperatures of 25, 50, 100, 150 200, 210, 220, 230, 240, and 250°C under argon ("anoxic conditions") and under air ("oxygenic conditions") in a Leitz #1350 microscope heating-stage (accurate to ± 0.5°C).

2) Ten 0.1 gm samples of apochlorotic Lyngbya filaments cleared both of chlorophyll (by extraction with acetone/water 80:20 volume) and of phycobiliproteins (by extraction with pH 7 aqueous phosphate buffer), were heated for thirty minutes each at temperatures identical to those in Experiment 1, under argon in the same heating-stage. Empty sheaths and filaments cleared of chlorophyll but containing phycobiliproteins were heated under similar conditions.

3) Twenty 0.5 ml samples of concentrated chlorophyll a, extracted from Lyngbya filaments with acetone/water (80:20 by volume), were heated for thirty minutes each at temperatures identical to those in Experiment 1, under argon and under air in the same heating-stage.

Long-term heating experiments

4) Seventy-two 2 gm samples of Lyngbya filaments were sealed in glass capsules, half of the samples under nitrogen ("anoxic conditions") and half under air ("oxygenic conditions") and heated in a sand bath for 1, 2.5, 5, 10, 25, 50, 100, 175, 250, 500, 1000, and 2000 hours at temperatures of 100, 125, and 150°C (accurate to ± 5°C).

5) Two 0.7 gm samples of Lyngbya filaments were heated in an oven under flowing nitrogen for 45 and 300 hours each at a temperature of 150°C (accurate to ± 5°C).

6) One 0.7 gm sample of Lyngbya filaments was heated in an oven at a temperature of 150°C for 120 hours under flowing nitrogen; the sample was then heated in the same oven at 150°C for 130 hours under flowing air.

7) Twelve samples of Holocene sediments from the Tanner Basin (about 220 km west of La Jolla, California), weighing approximately 50 gm each, were sealed with their interstitial water in glass capsules and heated in an oven at temperatures of 25, 65, 100, and 150°C for 170, 770, and 1775 hours. The samples were prepared by homogenizing the top meter of sediment from a single core (see AIZENSHTAT, in press, for detailed discussion).

Pigment identifications were made by visible spectral

analyses of benzene, benzene/methanol (70:30 by volume), and 10% KOH/methanol extracts, using a Cary 15 UV-visible Spectrophotometer. Mass spectral analyses of ether-soluble pigments for Experiment 6 were conducted on an AEI MS-9 Mass Spectrometer. Elemental analyses of carbon and hydrogen in thermally altered Lyngbya filaments were carried out by H. King of the UCLA Department of Chemistry, Microanalytical Laboratory. Infrared spectra of KBr-embedded, thermally treated algae were measured on a Perkin-Elmer 137B Infracord Spectrophotometer. Determination of algal morphology, of filament and sheath color, and of chlorophyll color resulting from thermal treatment were made by microscopic inspection and by comparison of color photomicrographs taken at both low- (10X objective) and high-magnification (100X, oil-immersion objective) immediately following each experiment.

RESULTS AND DISCUSSION

Thermal alteration of algae under oxygenic conditions

Under oxygenic conditions (i.e., in the presence of air), algae heated for both short and long periods of time at temperatures ranging from 25 to 300°C (Experiments 1 and 4 plus one thirty-minute heating experiment at 300°C) exhibited the following sequential color changes: blue-green/yellow-green/yellow-orange/red-orange/red-opaque. These hues represent the color of the entire filament (including both

the internal cellular trichome and the external encompassing sheath); transitions in color may therefore reflect thermal alteration of both intracellular (pigments, cytoplasm) and extracytoplasmic components (cell walls and sheaths). As is shown in Fig. 1, the sequential color transformations are both time- and temperature dependent; the same transformations observed at relatively low temperatures and long periods of time occur in shorter periods of time at higher temperatures.

Correlative with these irreversible changes in overall filament color were changes in the morphology, color and chemistry of the encompassing sheaths. The sheaths of fresh Lyngbya are broad, hyaline and, in plane-polarized light, are highly birefringent, a feature reflecting the ordered, micellar arrangement of their component polysaccharides and pectic substances (LANG, 1968). This birefringence and the hyaline sheath color were virtually unaffected during the blue-green/yellow-green/yellow-orange transitions. With further heating, however, and attainment of the red-orange condition, sheaths had become amber to orange in color and birefringence had somewhat decreased. With attainment of the red-opaque condition, sheaths had become ruby red, thin, closely adpressed against trichomes, and nonbirefringent. This loss of birefringence presumably reflects a loss of micellar order resulting from thermal condensation reactions which apparently involve introduction of chromophoric

carbon-carbon double bonds. These observations and the occurrence of a similar alteration sequence for the algal cell walls suggest that under severe conditions (i.e., above the red-orange/red-opaque transition), total filament color may be influenced by colored products resulting from thermal condensation and aromatization (Fig. 2) of sheath and cell wall material. Under less severe conditions, however, sheaths and cell walls are essentially hyaline; thus, filament color under these conditions must be determined primarily by colored intracellular components. As is shown in Fig. 1, color changes observed during heating of neat chlorophyll, under oxygenic conditions at temperatures ranging from 25 to 250°C (Experiment 3), are in good agreement with those observed in similarly treated whole algae. Thus, it seems likely that algal color changes occurring prior to the yellow-orange/red-orange transition and, possibly, prior to the red-orange/red-opaque transition, chiefly result from thermal alteration of intracellular chlorophyll and its derivatives.

The slope of the blue-green/yellow-green transition isochrome in the Arrhenius plot shown in Fig. 1 corresponds to an activation energy of approximately 13,700 cal/mole; the slopes of the non-green transition isochromes correspond to activation energies in the range of 33,500-38,000 cal/mole. Interestingly, these latter values are comparable to

activation energies of approximately 30,000 cal/mole required for introduction of carbon-carbon double bonds to a variety of organic compounds (FIESER and FIESER, 1963; COULSON and STEWART, 1964). Although it is evident from the infrared spectra shown in Fig. 2 that aromatization does, in fact, occur during the thermal alteration sequence, it must be emphasized that the observed color changes almost certainly result from a complex of chemical reactions; the apparent activation energies indicated in Fig. 1 presumably reflect a net effect rather than energies required for any individual reaction.

Thermal alteration of algae under anoxic conditions

Under anoxic conditions (i.e., under argon or nitrogen), algae heated for both short and long periods of time at temperatures ranging from 25 to 250°C (Experiments 1 and 4) exhibited the following sequential changes: blue-green/yellow-green/yellow-orange. The time-temperature relationships of these color changes are shown in the form of an Arrhenius plot in Fig. 3. During these experiments, the algal sheaths gradually became altered from hayline to slightly yellow but exhibited no morphologic change and retained strong birefringence. Similarly, little change was observed in empty sheaths or in cell walls of cleared, apochlorotic trichomes during short-term heating experiments (Experiment

2). It seems probable, therefore, that the relatively minor color changes occurring during anoxic heating of entire filaments primarily reflect thermal alteration of intracellular pigments. As is shown in Fig. 3, the color of neat chlorophyll remains essentially unchanged up to 250°C during short-term anoxic heating experiments (Experiment 3), a result in good agreement with the color sequence observed in similarly treated entire filaments.

The slope of the blue-green/yellow-green transition isochrome in Fig. 3 corresponds to an activation energy of approximately 3,850 cal/mole; that of the yellow-green/yellow-orange transition isochrome corresponds to an activation energy of approximately 30,000 cal/mole. Differences between these net activation energies and those obtained under oxygenic conditions (Fig. 1), especially of the respective blue-green/yellow-green transition isochromes, suggest that redox potential may be important in determing which of several possible mechanisms are involved in at least the initial stages of thermal alteration of intracellular chlorophyll. The major differences between these results and those of the comparable suite of experiments conducted under oxygenic conditions, however, are evident at relatively high temperatures; anoxic conditions apparently retard those chemical reactions that result in severe alteration of sheaths, cell walls, and intracellular chlorophyll in the presence of oxygen.

Paleobiologic implications

Thus, alterations of two distinct types of organic material appear to be responsible for the sequential color changes observed in entire Lyngbya filaments subjected to thermal treatment. Under relatively mild or moderate conditions, color changes apparently result primarily from alteration of intracellular components, chiefly chlorophyll and its derivatives; under more severe conditions, however, color changes resulting from pigment alteration appear to be augmented by changes resulting from alteration of sheath and cell wall chemistry.

Inasmuch as palynomorphs, in general, lack the photosynthetic and accessory pigments present in Lyngbya, the color changes here observed in intact filaments cannot be considered strictly comparable with those occurring during thermal alteration of pollen and spores. As is shown in Table 1, however, palynomorph color changes [as reflected in the "State of Preservation Index" of CORREIA (1971) and the "Thermal Alteration Index" of STAPLIN (1969)] do appear to be roughly similar to changes observed in Lyngbya sheaths and cell walls. Further, processes producing these changes in extracytoplasmic material (thermal condensation reactions and aromatization resulting in an increase in percentage of fixed carbon and in C/H ratio) are apparently rather comparable to those involved in the alteration of palynomorphs (see

CORREIA, 1971). Chlorophyllous plant cells and tissues may be expected to more nearly parallel the alteration sequence observed for intact Lyngbya filaments. For example, it could be suggested based on Figs. 1 and 3 that fossilized algal or higher plant cells that had experienced mild thermal regimes might remain within the yellow-green stability field for as long as 10^7 or perhaps 10^8 years. Apparently the oldest known plant fossils retaining such color are leaves preserved in middle Eocene brown coals, approximately 4.5×10^7 years in age (DILCHER et al., 1970); the occurrence of yellow-green chlorophyll derivatives in these fossils, preserved in sediments having "a history of low temperatures" (DILCHER et al., 1970, p. 1447), seems consistent with the time-temperature relationships here observed.

Results of heating experiments with Holocene sediments from the Tanner Basin are discussed in detail elsewhere (KAPLAN et al., work in progress). Of interest here, however, are color changes observed in the extractable tetrapyrrole fraction of Tanner Basin samples heated with their interstitial water in sealed glass capsules (Experiment 7). The sediment has an estimated age of approximately 10,000 years (EMERY, 1960). The extractable tetrapyrrole fraction of the unheated sediments is yellow-green in color and contains chlorins, characteristics consistent with the data summarized in Figs. 1 and 3. During heating, however, this fraction was observed

to change color from yellow-green to orange-brown; the color changes occurring in this experiment are summarized in Fig. 4, compared with the combined results from Figs. 1 and 3. Although the sediment and algal colors are not precisely correlative, the disappearance of green color from the sediment extracts seems to agree well with the disappearance of green color from the algae heated under both oxygenic and anoxic conditions. These results suggest that color changes similar to those observed in the algal and chlorophyll heating experiments can occur in thermally treated natural sediments as well.

Thermal alteration of blue-green algal chlorophyll

As is discussed above, the sequential color changes observed in entire Lyngbya filaments subjected to relatively mild or moderate thermal conditions appear to result primarily from alteration of intracellular pigments. A series of experiments was therefore conducted to characterize this alteration sequence and to determine whether the degradation pathways for intracellular chlorophyll differ significantly from those described for free, extracellular chlorophyll and its derivatives (ORR et al., 1958).

Neat blue-green algal chlorophyll, heated for thirty-minute periods under anoxic conditions (Experiment 3) remained green up to 250°C; visible spectra of the unsublimed,

benzene-soluble pigment are indicative of chlorophyll *a* (Fig. 5). During similar experiments under oxygenic conditions, neat chlorophyll changed color from green to yellow-orange, to red-orange; as shown in the visible spectra in Fig. 5, the trend toward total absorption during this sequence suggests that with attainment of the yellow-orange condition at about 200°C, the pigment began to self-polymerize (Fig. 5).

The pathways of thermal degradation of intracellular chlorophyll differ significantly from those of similarly treated neat chlorophyll. In Fig. 6 are shown visible spectra of pigments extracted from algae heated for thirty-minute periods under anoxic conditions (Experiment 1). Phycobiliproteins were not detected in any of these spectra, presumably due to masking by the more intense chlorophyll bands at low temperatures and because of their thermal lability at high temperatures; during heating of algal filaments cleared of chlorophyll but still containing phycobiliproteins (Experiment 2), the lavender color indicating the presence of phycocyanin and phycoerythrin disappeared immediately upon exposure to temperatures above 100°C. With regard to the intracellular degradation of chlorophyll, the spectra shown in Fig. 6 indicate that chlorophyll *a* remained unchanged up to 150°C; that at about 200°C a portion of the chlorophyll had apparently been altered to pheophytin *a*; and that above 200°C only pheophytin *a* could be detected. Although red-brown pigment was extracted from

algae heated at 250°C, the visible spectrum of this extract showed broad, essentially featureless, absorption (Fig. 6); neither chlorophyll nor pheophytin could be identified.

Spectral results similar to those shown in Fig. 6 for short-term algal heating experiments were also obtained during long-term heating experiments (Experiments 5 and 6). Visible absorption spectra of pigments extracted from algae heated for 45 and 300 hours at 150°C under nitrogen (Experiment 5) are indicative of pheophytin a (Fig. 7, Parts 1 and 2). Spectra of pigments from algae heated at 150°C for 120 hours under nitrogen and subsequently for 130 hours under air (Experiment 6) show only total absorption (Fig. 7, Part 3). Thus, during both short-term anoxic heating at high temperatures (30 minutes, 250°C) and long-term anoxic-oxygenic heating at moderate temperatures (250 hours, 150°C), intracellular chlorophyll was degraded to pheophytin and ultimately to a colored material that could not be characterized adequately by visible absorption spectroscopy. This evidence, together with the well-established chemical affinity between porphyrins and biological macromolecules (FALK, 1964, p. 24-25) and the known physical association of chlorophyll and polymeric membrane systems (thylakoids) in blue-green algal cells (LANG, 1968), suggests that the unidentified pigments produced in these experiments might actually constitute, in part, pigment-polymer complexes, presumably formed by thermochemical grafting of chlorophyll derivatives onto cellular membranes.

In Experiment 6, the amount of ether-soluble pigment recovered from base extracts of the algae (see Fig. 7, Part 3A) was sufficient for analysis by mass spectroscopy to determine whether polymeric material might be present. As is evident in Fig. 8, the resulting mass spectrum shows a polymeric fragmentation pattern with ions in excess of 700 m/e. Thus, it seems apparent that pigment-polymer complexes are indeed produced during thermal degradation of intracellular chlorophyll. Attempts to dissociate such pigment-polymer complexes by acid hydrolysis were unsuccessful. However, as can be seen in Fig. 7, base extraction (using 10% KOH/methanol) released five to ten times more pigment and pigment-polymer complex from algae heated at 150°C for prolonged periods of time (Experiments 5 and 6) than previous extractions of the same algae using only benzene/methanol (70:30 by volume). These results suggest that chemical bonding through base-saponifiable (e.g., ester and related) linkages may be a major grafting mechanism responsible for formation of pigment-polymer complexes of this type.

Thermal production of pigment-polymer associations seems further evidenced by observations, in both the algal and sediment heating experiments, that with increasing time and temperature pigment fractions become increasingly soluble in aqueous media and decreasingly soluble in organic solvents. In algae, these solubility characteristics presumably result

from the relatively high proportion of polar functional groups on the polymeric portion (e.g., polysaccharides, polypeptides, peptidoglycans) of the pigment-polymer complex; in sediments, this behavior presumably reflects grafting of pigments onto relatively polar humic and fulvic acids.

Geochemical implications

In general, chlorophyll first enters the sedimentary environment as a component of dead plant cells. As a result of biologic activity, the vast majority of these pigments are recycled through the biosphere. Of that fraction which escapes such recycling and is thus potentially preservable in the geologic record, some portion occurs as free pigments, "leached" from plant cells during deposition. This portion, however, apparently represents less than a quarter of that occurring in association with suspended solids in the water column and an even smaller fraction of that occurring in the underlying sediments (PEAKE et al., 1972). It seems apparent, therefore, that the majority of preservable, sedimentary chlorophyll and chlorophyll derivatives is initially deposited in a particulate state; the bulk of such pigments probably occurs within decaying plant cells and tissues.

Based on studies of chlorophyll and chlorophyll derivatives occurring in marine sediments off southern

California, ORR et al. (1958, p. 953) have postulated a set of degradation pathways leading from chlorophyll to porphyrins of the type found in petroleum and sedimentary rocks. These pathways, however, differ from the degradation sequences here observed for chlorophyll occurring within thermally treated algal cells. Specifically, our results suggest that the major intracellular derivative is pheophytin which subsequently becomes grafted onto cellular polymers; further degradation, for example to pheophorbides, phylloerythrins, or porphyrins, was not observed. Thus, although the degradation pathways suggested by ORR et al. (1958) are apparently valid for extracellular chlorophyll, it appears to us likely that chlorophyll and its derivatives entering the geologic record as components of intact or partially degraded plant cells may become grafted onto cellular polymers.

Formation of pigment-polymer complexes and their subsequent geochemical degradation may, therefore, be important factors affecting the distribution of chlorins and porphyrins in sediments and sedimentary rocks. Degradation of such pigment-polymer complexes might release free porphyrins or porphyrins still complexed with oligomeric fragments of the original polymer. Such a mechanism could account for the formation of anomalously "heavy porphyrins" (BLUMER and RUDRUM, 1970) as well as some reported porphyrin-peptide complexes (HODGSON et al., 1969, 1970). Hydrogenation and

cleavage of such complexes under reducing conditions could release secondary synthetic chlorins of the type identified by BLUMER and OMENN (1961). Alternatively, pigment-polymer complexes might become incorporated into the humic and fulvic acid fractions of sedimentary organic matter or become bound into the insoluble particulate "kerogen" fraction. Pigment grafting onto materials of this type might explain observations of BROWN et al. (in press) that the amount of chlorins extractable from Holocene cores from Saanich Inlet decreased by a factor of fifteen between the sediment-water interface and a depth of 3.5 meters, without yielding detectable porphyrins. Similar pigment grafting followed by cleavage and release of extractable pigments might also explain results obtained by AIZENSHTAT et al. (in press) who found, in a Pleistocene core from JOIDES Hole J-26, that although chlorin content decreased steadily with depth, porphyrins were not extractable until a depth of 478 meters, at which level chlorins were no longer detected.

A schematic diagram summarizing the reaction pathways discussed above is shown in Fig. 9.

CONCLUSIONS

1) Sequential color changes observed during thermal degradation of Lyngbya result from two processes: (i) transformations of

intracellular pigments and (ii) alteration of extracytoplasmic components (sheaths and cell walls). These irreversible color changes are comparable to those occurring during eometamorphism of plant tissues and palynomorphs and are both time- and temperature-dependent. In addition to pigment changes, thermal degradation of algae results in dehydration (i.e., formation of anhydrides), aromatization, and increases in C/H ratios and in the percentage of fixed carbon.

2) Extrapolations based on Arrhenius plots of the time-temperature relationships of the observed color changes in algae and algal chlorophyll are in good agreement with changes observed in extracts of Holocene and Tertiary sediments. The experimental data thus appear to have geologic applicability, and they seem to indicate clearly that time is an important parameter affecting the color and fidelity of preservation of plant microfossils.

3) Pigments deposited in sediments as components of intact or partially degraded plant cells may become grafted during diagenesis onto a variety of cellular polymers, at least in part, apparently, through ester and related linkages. It appears possible, and is perhaps likely, that formation of such pigment-polymer complexes is a major diagenetic process affecting the types and distribution of pigments in sediments and sedimentary rocks.

4) Once formed, geochemical alteration of pigment-polymer complexes may release free chlorins, free porphyrins, or pigments attached to oligomeric fragments of the original polymer. Alternatively, the pigment-polymer moiety may become further complexed and incorporated into the humic and fulvic acid fractions, or into the insoluble "kerogen" fraction, of sedimentary organic matter.

Acknowledgements--We thank Mrs. L. Flesché for excellent assistance. Coring in Tanner Basin was funded by R. Hessler (Scripps Vessel R/V Alexander Agassiz) and the sediments were collected by E. K. Kalil and R. Sweeney. This study was supported by the National Science Foundation, Earth Sciences Section, NSF Grant GA-23741, and by NASA Grants NGR 05-007-221 and NGR 05-007-292. Use of the mass spectrometer (Department of Chemistry, UCLA) was funded by NSF Grant GP 3672.

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Table 1

THIS REPORT		CORREIA, 1971			STAPLIN, 1969		WILSON, 1971		THIS REPORT	
Algal Colors		"State of Preservation Index"			"Thermal Alteration Index"		"Color and Preservation Scale"		Carbon and Hydrogen Determinations	
Total Filament	Sheaths, Cell Walls	Palynomorph Color	Amorphous Organic Matter Color	Index	Palynomorph Color	Index	Palynomorph Color	Approximate Fixed Carbon Percentage	Fixed Carbon Percentage	C/H Ratio
blue-green	clear	-	-	~~0~~	-	~~0~~	-	-	43.4[a]	6.8[a]
yellow-green	clear	-	-	~~0~~	-	~~0~~	-	-	-	-
yellow-orange	light yellow	clear, yellow	clear, yellow to red	1	fresh, yellow	1	yellow	50 - 55	45.8[b]	7.7[b]
red-orange	yellow-orange	orange	reddish orange	1-2	yellow	1-2	yellow	50 - 55	-	-
red-opaque	red	orange to reddish	reddish brown	2	brownish yellow	2	yellow, light brown	50 - 55	48.9[c]	8.7[c]
-	-	brown	brown	3	brown	3	light brown, dark brown	55 - 65	-	-
-	-	brown, black	black	4	brown	4	light brown, dark brown	55 - 65	-	-
-	-	brown, black	black	5	brown	5	dark brown, black	70	-	-

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a--Determinations made ~~of~~ or fresh Lyngbya

b--Determinations made on Lyngbya heated at 150°C for 2000 hours under nitrogen.

c--Determinations made on ~~Lyngbya~~ heated at 150°C for 2000 hours under air.

FIGURE DESCRIPTIONS

FIG. 1 Time-temperature relationships of heat-induced color changes in Lyngbya (circles) and neat chlorophyll a (squares) under oxygenic conditions (Experiments 1, 3, and 4); ——— indicates experimentally determined color changes; ------ indicates extrapolations; —·—·—· indicates reference activation energy curves from Abelson (1959); Y-O indicates yellow-orange; R-O indicates red-orange.

FIG. 2 Infrared spectra of whole algae (0.002 gm of Lyngbya pressed into 0.25 gm KBr pellets) thermally degraded under oxygenic conditions, representing blue-green (B-G), yellow-green (Y-G), yellow-orange (Y-O), and red-orange (R-O) color stages. Absorption increases in the 1540 and 1800 cm^{-1} regions are interpreted as indicating increases in aromaticity and dehydration (i.e., anhydride formation), respectively.

FIG. 3 Time-temperature relationships of heat-induced color changes observed in Lyngbya (circles) and neat chlorophyll a (squares) under anoxic conditions; ——— indicates experimentally determined color changes; ------ indicates extrapolations; —·—·—· indicates reference activation energy curves from Abelson (1959).

FIG. 4 Time-temperature relationships of heat-induced color changes in the extractable tetrapyrrole fraction of Tanner Basin sediments (circles) compared with the color transition isochromes from Fig. 1 (solid lines) and Fig. 2 (dashed lines). B-G indicates blue-green; Y-G indicates yellow-green; Y-O indicates yellow-orange; R-O indicates red-orange; R-Op indicates red-opaque; O-Br indicates orange-brown.

Fig. 5 Visible absorption spectra (in benzene) of neat chlorophyll heated for thirty-minute periods under anoxic (solid lines) and oxygenic (dashed lines) conditions at each of the indicated temperatures.

FIG. 6 Visible absorption spectra (in ether) of pigments extracted from Lyngbya after heating for thirty-minute periods under anoxic conditions at each of the indicated temperatures.

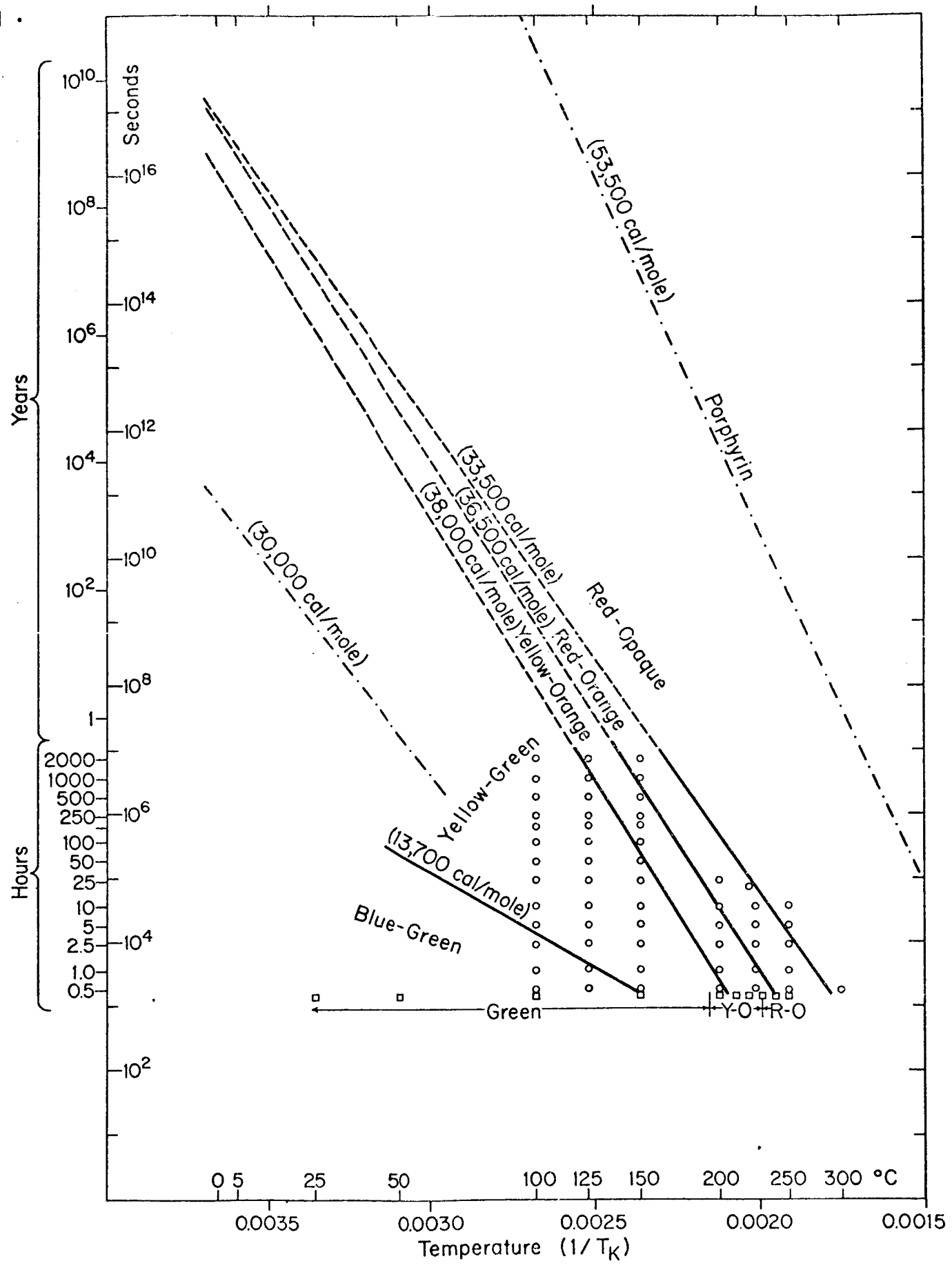
FIG. 7 Comparison of visible absorption spectra of benzene/methanol (70:30 by volume) extractable pigments (B) and 10% KOH/methanol extractable pigments (A) from Lyngbya heated under the following conditions: 1 = 45 hours at 150°C under nitrogen (Experiment 5); 2 = 300 hours at 150°C under nitrogen (Experiment 5); 3 = 120 hours at

150°C under nitrogen followed by 130 hours at 150°C under air (Experiment 6). Spectral solvents: Spectra 1A, 2A, and 3A in 10% KOH/methanol; Spectrum 1B in methanol; Spectra 2B and 3B in benzene.

FIG. 8 Mass spectrum (70 ev 2.4 kV, 270 μA, 280°C probe temperature) showing polymeric fragmentation pattern of the ether-soluble fraction of base-extracted pigments from Experiment 6; the visible absorption spectrum of these pigments is shown in Fig. 7, Spectrum 3A.

FIG. 9 Schematic diagram summarizing suggested relationships between pigment-polymer compexes and diagenetic processes possibly affecting the types and distribution of chlorophyll-derived pigments in sediments and sedimentary rocks.

Fig. 1 •


Seconds
Years
Hours
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10^{14}
10^{6}
10^{12}
10^{4}
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10^{8}
1
2000
1000
500
250
10^{6}
100
50
25
10
5
2.5
10^{4}
1.0
0.5
10^{2}
(53,500 cal/mole)
Porphyrin
(33,500 cal/mole)
(36,500 cal/mole) Red-Orange
(38,000 cal/mole) Yellow-Orange
Red-Opaque
(30,000 cal/mole)
Yellow-Green
(13,700 cal/mole)
Blue-Green
Green
Y-O
R-O
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0.0035 0.0030 0.0025 0.0020 0.0015
Temperature (1/ T_K)

Fig.2

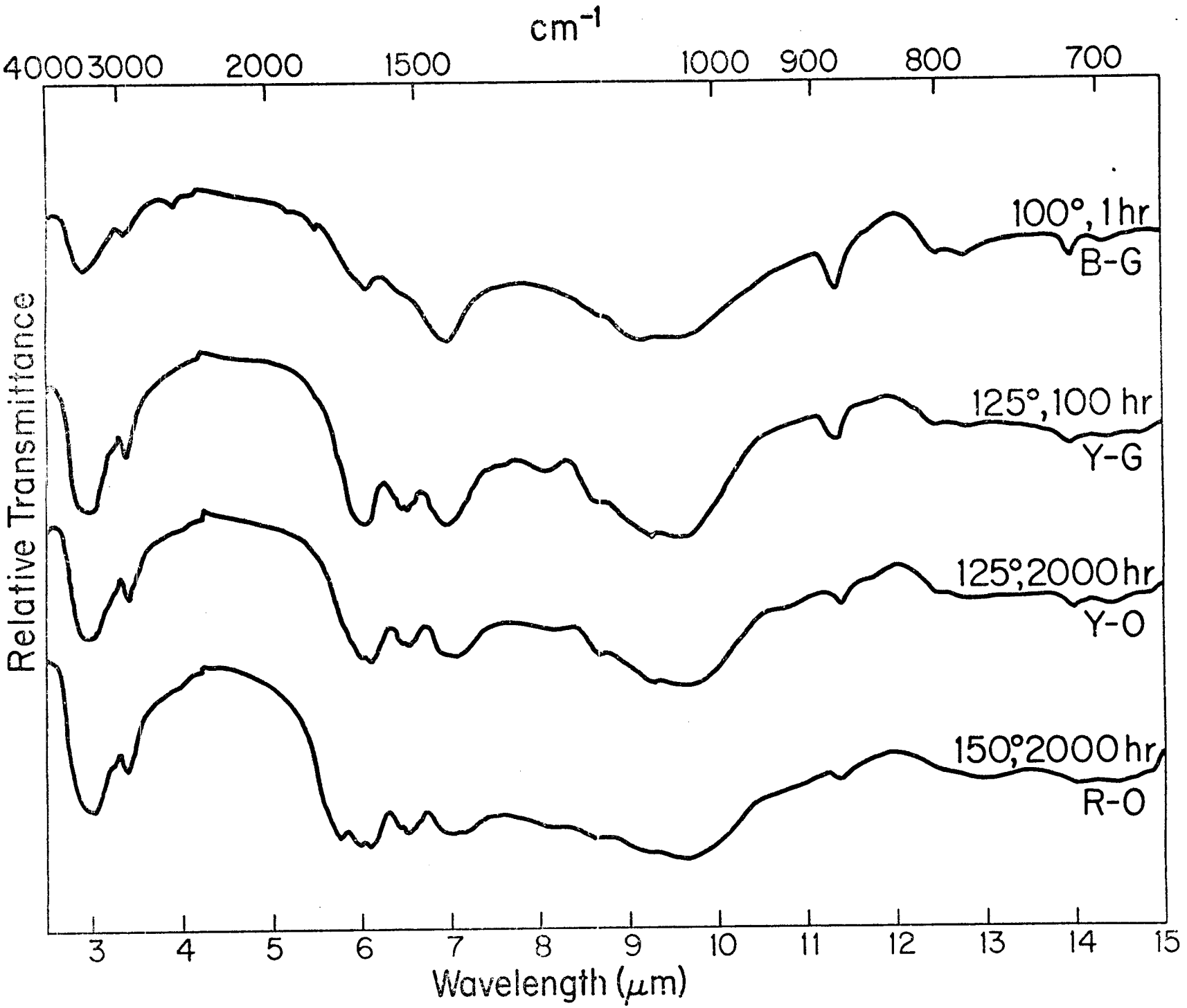
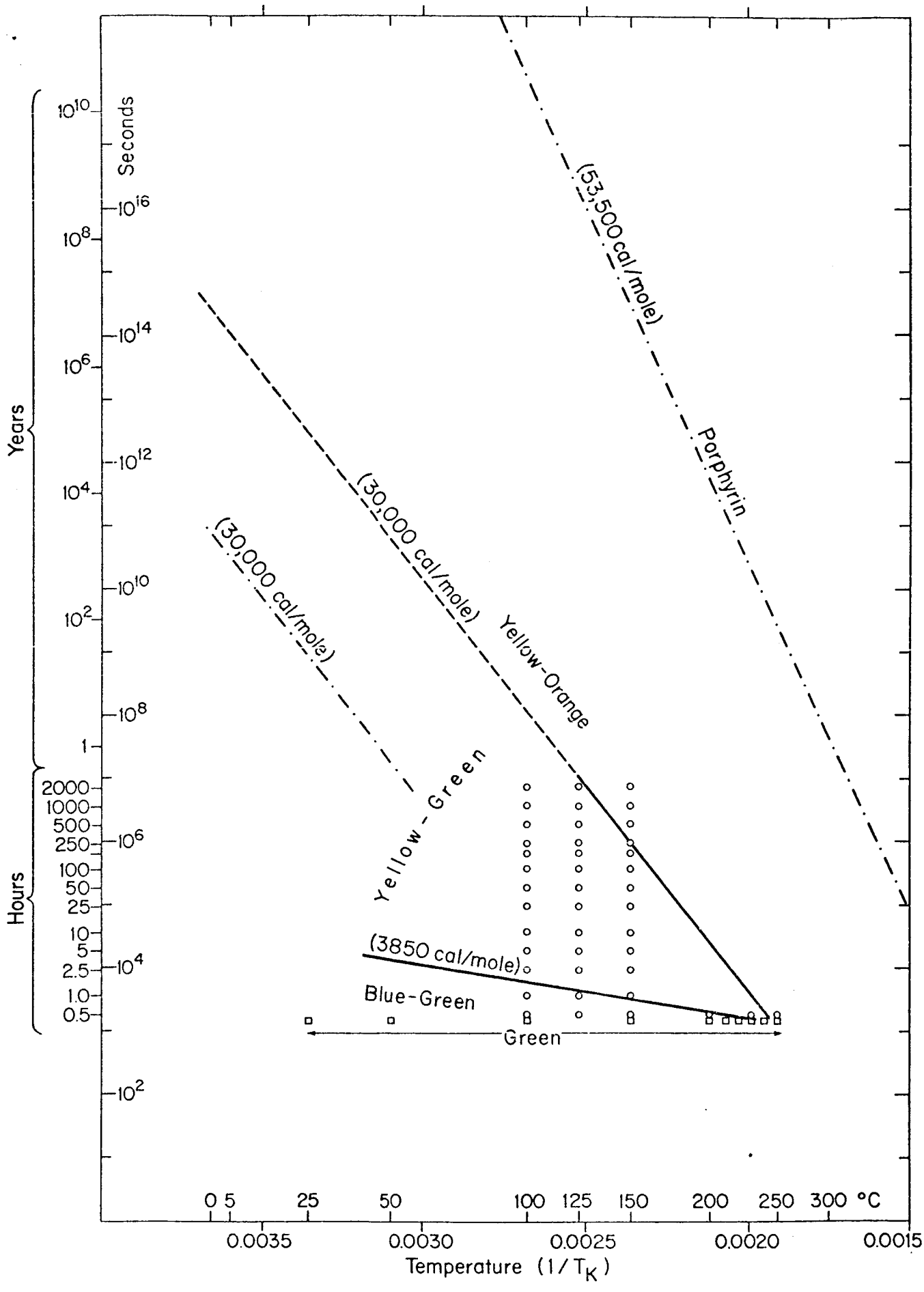

cm-1
4000 3000 2000 1500 1000 900 800 700
100°, 1 hr
B-G
125°, 100 hr
Y-G
125°, 2000 hr
Y-O
150°, 2000 hr
R-O
Relative Transmittance
3 4 5 6 7 8 9 10 11 12 13 14 15
Wavelength (μm)

Fig.3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10^{2}
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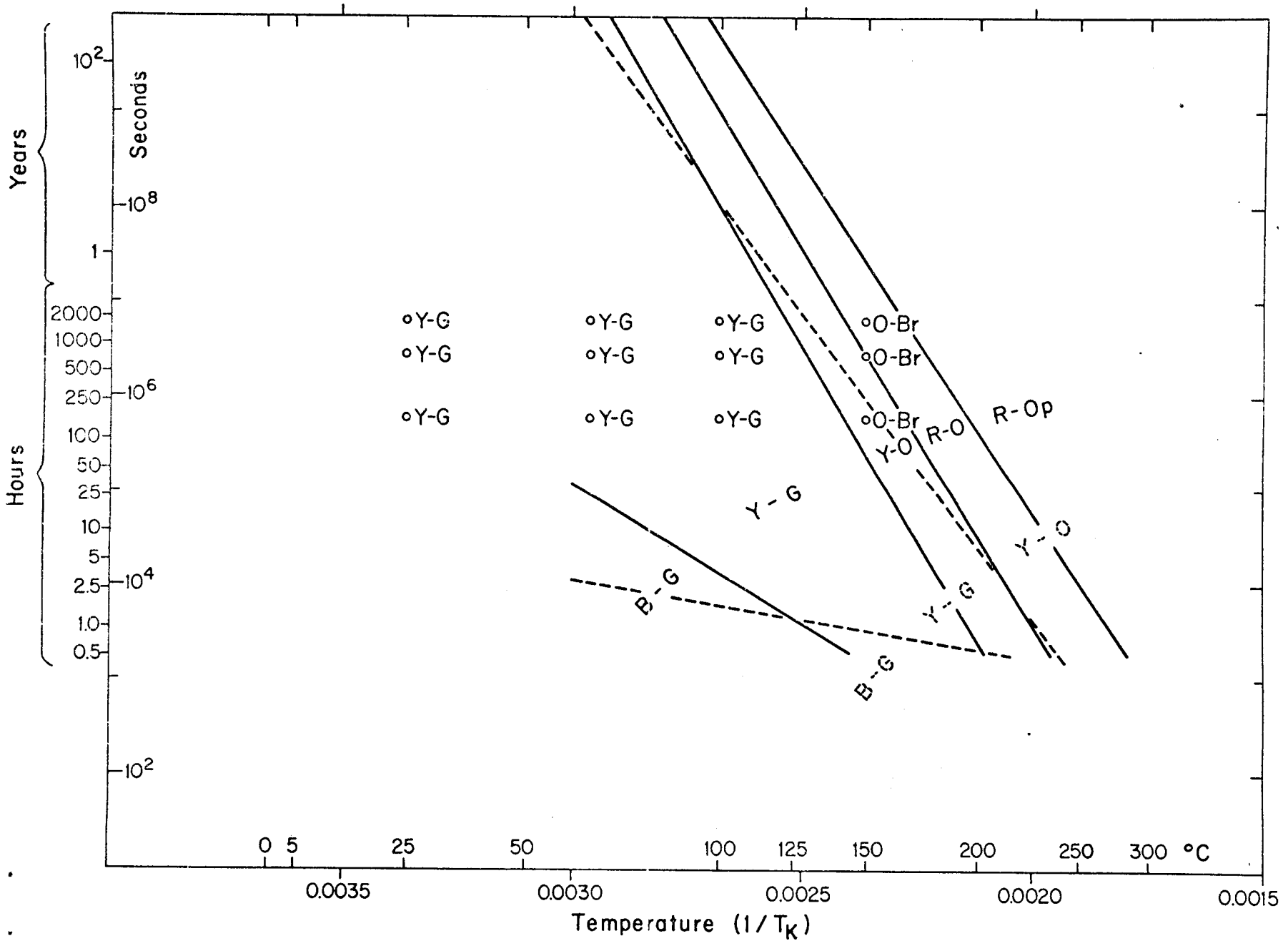
2000
1000
500
250
100
50
25
10
5
2.5
1.0
0.5
10^{16}
10^{14}
10^{12}
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
(53,500 cal/mole)
Porphyrin
(30,000 cal/mole)
Yellow-Orange
(30,000 cal/mole)
Yellow-Green
(3850 cal/mole)
Blue-Green
Green
0 5 25 50 100 125 150 200 250 300 °C
0.0035 0.0030 0.0025 0.0020 0.0015
Temperature ($1/T_K$)

Fig.4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Hours
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10^8
1
2000
1000
500
250
10^6
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50
25
10
5
2.5
10^4
1.0
0.5
10^2
°Y-G
°O-Br
R-Op
R-O
Y-O
Y-G
B-G
0 5 25 50 100 125 150 200 250 300 °C
0.0035 0.0030 0.0025 0.0020 0.0015
Temperature (1/T_K)

Fig. 65

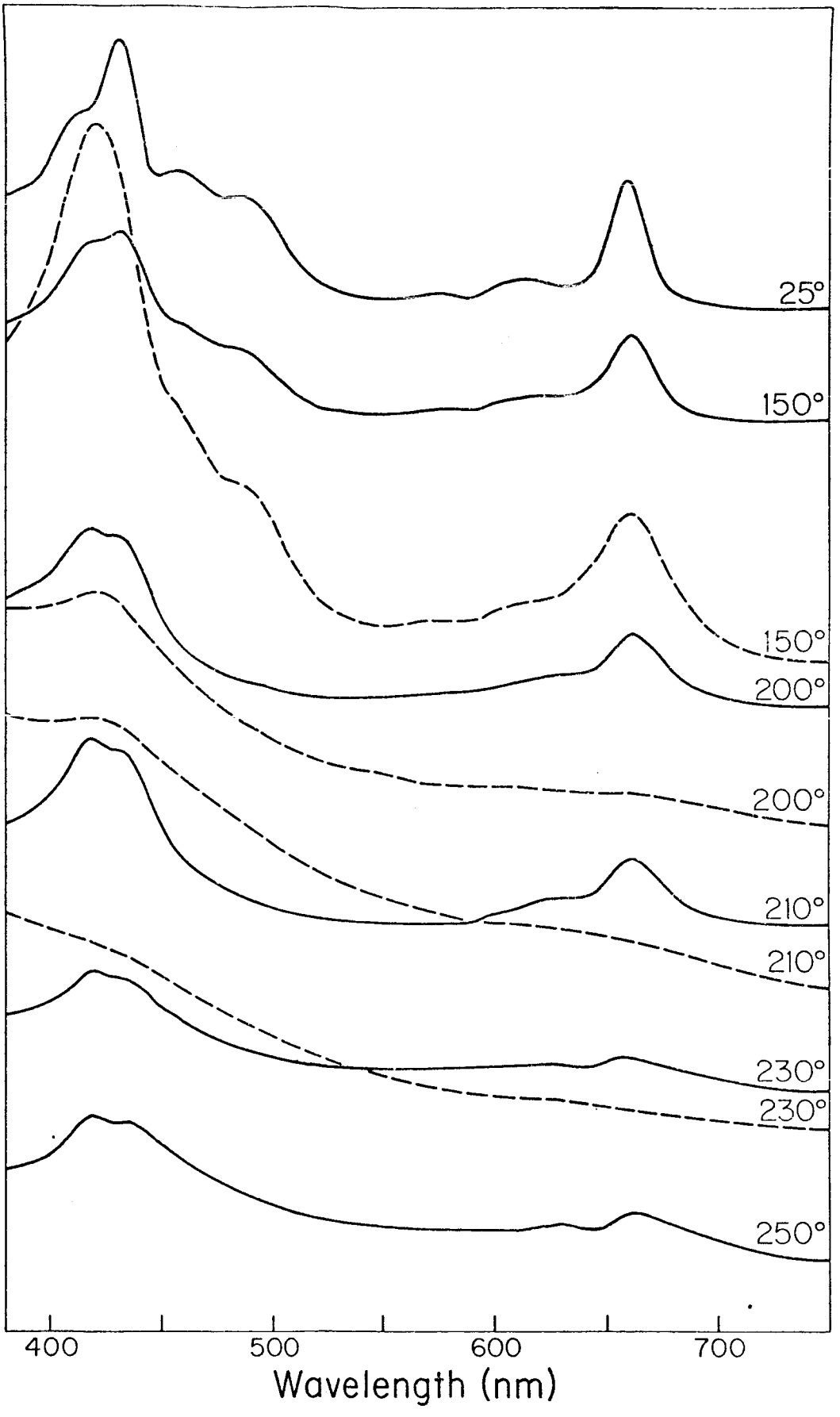

25°
150°
150°
200°
200°
210°
210°
230°
230°
250°
400
500
600
700
Wavelength (nm)

Fig. 6

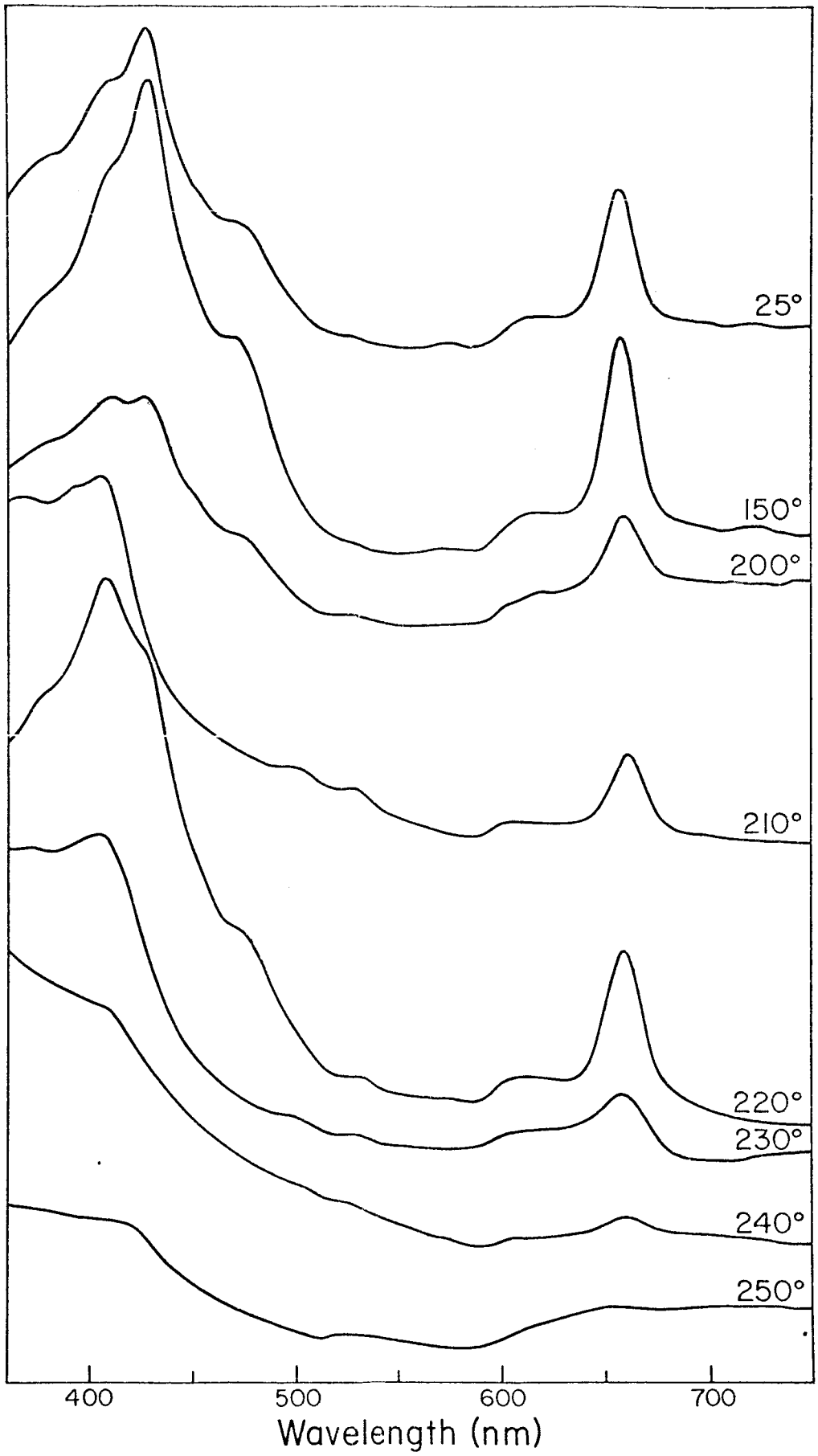


Fig. 7

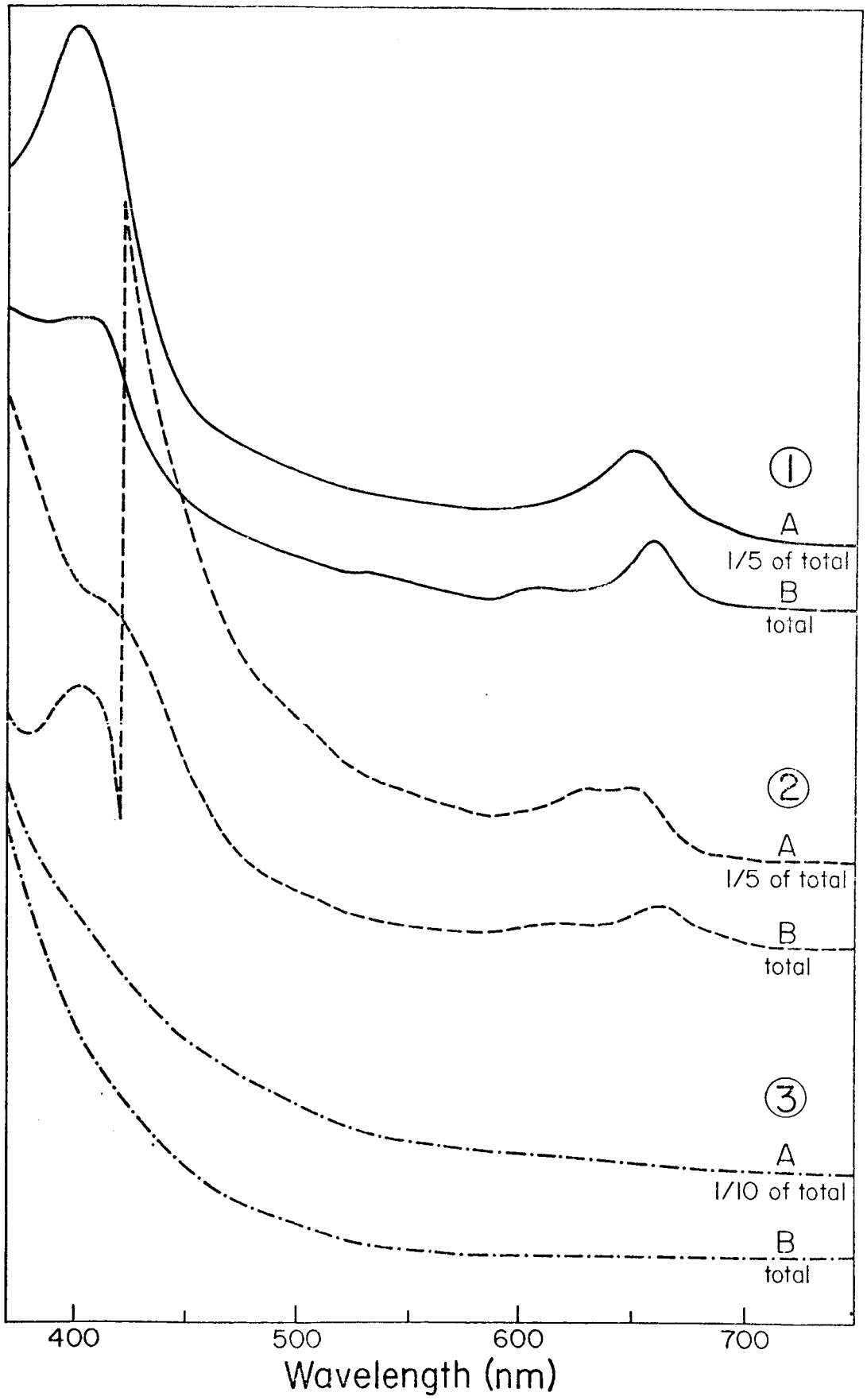

①
A
1/5 of total
B
total
②
A
1/5 of total
B
total
③
A
1/10 of total
B
total
400
500
600
700
Wavelength (nm)

Fig.8

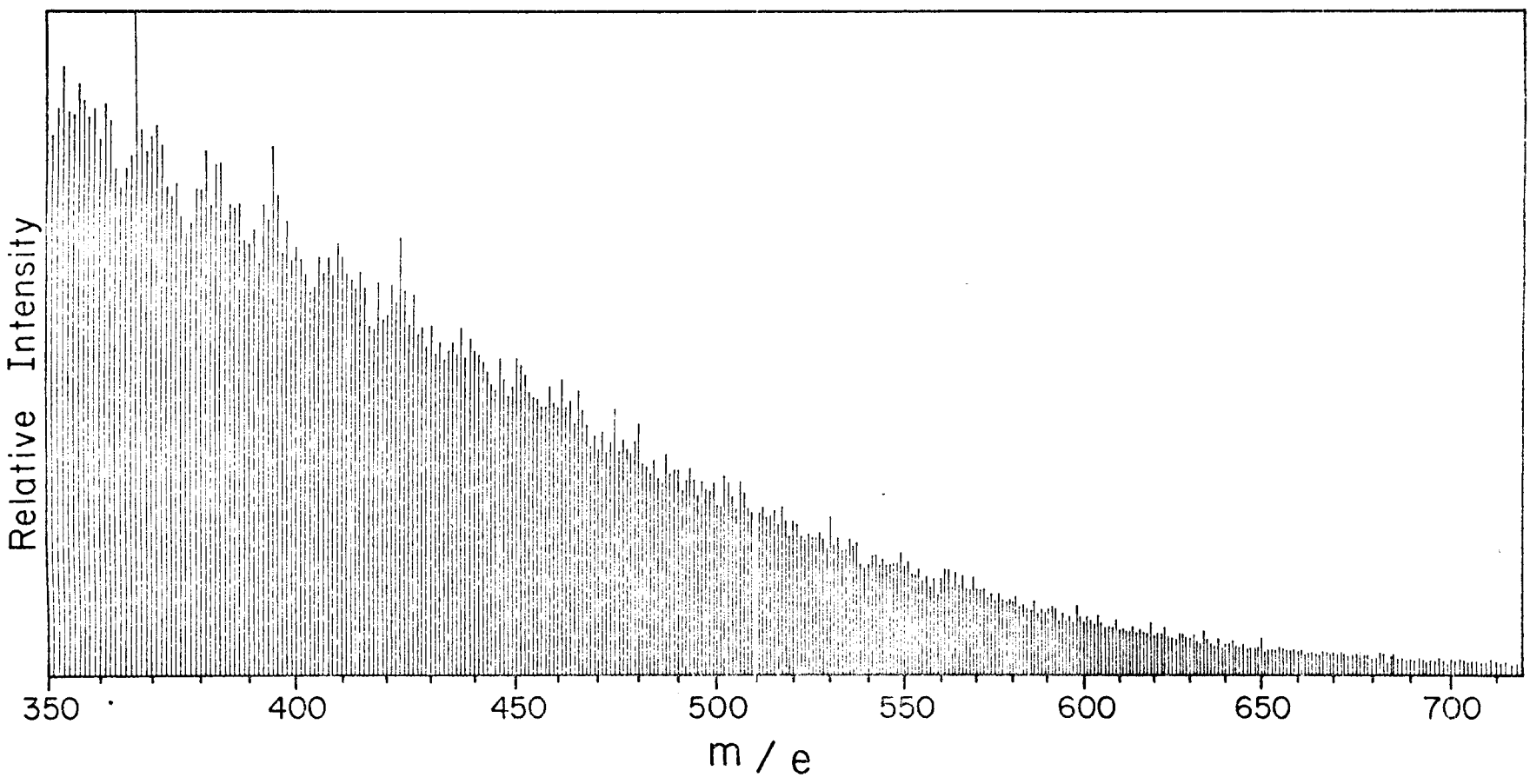
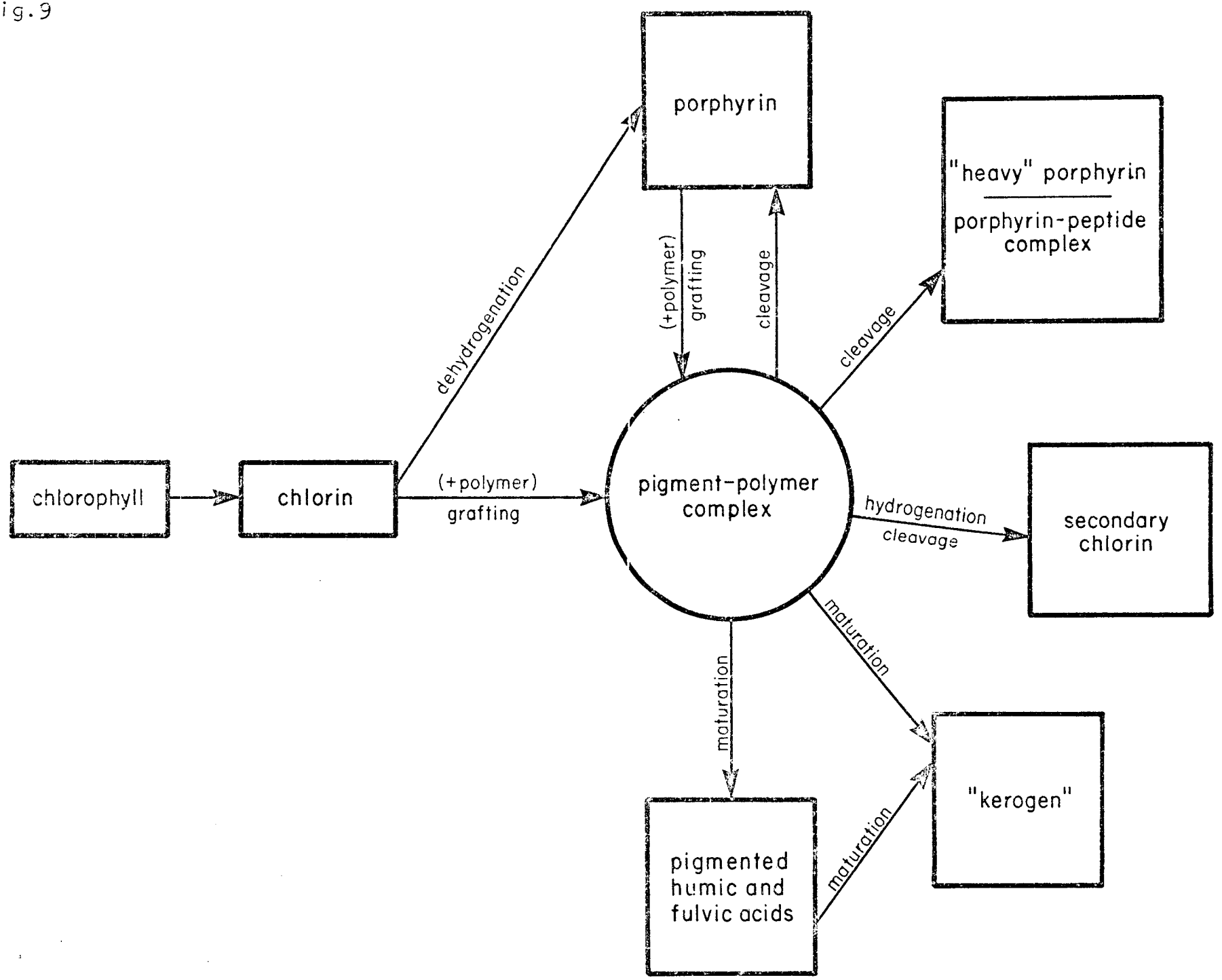


Fig.9


porphyrin
"heavy" porphyrin
porphyrin-peptide complex
dehydrogenation
(+polymer) grafting
cleavage
cleavage
chlorophyll
chlorin
(+polymer) grafting
pigment-polymer complex
hydrogenation cleavage
secondary chlorin
maturation
maturation
"kerogen"
pigmented humic and fulvic acids
maturation